Escherichia Coli Bacterin For The Reduction of Clinical Signs of Toxic Mastitis In Cattle

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While herd surveys have indicated that 95% of mastitis in dairy cattle is caused by gram-positive bacteria such as *Streptococcus agalactiae* and *Staphylococcus aureus*,[1,2] mastitis caused by coliform organisms is gaining in recognition. Coliforms causing mastitis include gram-negative, lactose-fermenting organisms of the family Enterobacteriaceae in the genera *Escherichia, Klebsiella* and *Enterobacter*.[2,3] Routine mastitis control measures such as udder washing, teat dipping and milking machine sanitation have reduced the incidence of subclinical mastitis caused by the cocci, but they have not affected the incidence of coliform infections.[2,4,5] Herds in which mastitis caused by *Str. agalactiae* and *S. aureus* is under control may still have problems with clinical mastitis caused by coliforms.[3,6]

Prevalence of *E. coli* Mastitis

Investigators believe that *E. coli* is the most significant organism causing coliform mastitis because of its wide presence in the barnyard or dairy environment,[3,7] and studies across the United States have supported this concept.[1,3,8,9]

In a 1982 study in New York, pathogens were isolated from udder quarters of cows affected with clinical mastitis which were not responding to intramammary antibiotic treatment. Forty-four percent (32 of 72) of the cultures resulted in the discovery of *Escherichia coli*.[1] In a group of studies on eight dairy herds in California, 63% of the 158 coliform organisms cultured from cases of clinical mastitis were *E. coli*, 10% were *Enterobacter aerogenes* and 11% were *Klebsiella pneumonia*,[3] suggesting that *E. coli* is the predominant cause of coliform mastitis in California. Similar results were obtained from a single herd in Iowa.[8]

In 1985-86, *Escherichia coli* was isolated year-round, and was the predominant organism isolated during the summer in a Wisconsin study.[9] *Escherichia coli* was the principal cause of mastitis of cows in early and late lactation, while nearly equal numbers of *E. coli, Corynebacterium pyogenes*, streptococci and staphylococci were isolated at parturition. In this study, 45.5% (66 of 145) of the cultures obtained from cows with mastitis and anorexia resulted in the growth of *E. coli*. It was noted that the coliforms caused a more watery milk, higher rectal temperatures, less udder swelling and increased weakness and anorexia than did other mastitis pathogens.

Another investigator[10] found that most udders were resistant to *E. coli* infections during nonlactating periods, and that they became susceptible just before parturition. Other reports concur that *E. coli* mastitis is most prevalent during early lactation, and declines during late lactation to an insignificant level in nonlactating periods.[2,6]

Pathogenesis and Clinical Signs of *E. coli* Mastitis

Clinical mastitis caused by *Escherichia coli* begins with phagocytosis of the bacteria with resulting endotoxin release. Absorption of endotoxin in the mammary gland is then believed to cause the subsequent inflammatory and systemic reactions.[2] Investigators have shown that endotoxin can be detected in the blood following intramammary infusion,[11] and that acute

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mastitis with systemic signs occurs after intramammary infusion of endotoxin obtained from *E. coli*.[12]

Inflammation in mastitis caused by *E.coli* is marked by an early leukopenia mostly due to mass migration of neutrophils, lymphocytes, monocytes and eosinophils to affected mammary quarters.[12-14] However, migration is not the only cause of the leukopenia, as one investigation showed that intravenous administration of endotoxin also causes leukopenia.[14] Mammary quarter inflammation is evidenced by increased redness and swelling, and by a more watery milk secretion containing clumps of cells. Other clinical laboratory findings include hyperglycemia,[12] hypercortisolemia[12,14] and hypocalcemia.[14] Systemic manifestations include fever, depression and anorexia.[2]

Escherichia Coli Bacterin Field Trial

Materials and Methods

Animals used in this field trial consisted of 39 pregnant Holstein heifers which were screened and selected on the basis of low blood titers to *E. coli* antibodies. The heifers had no known history of mastitis or other disease, or of vaccination against *E. coli*. They were randomly assigned to vaccinate (21 heifers) and control (18 heifers) groups.

The product tested was an *Escherichia coli* bacterin*, currently marketed to protect against calf scours. The 21 heifers in the vaccinate group received a single 2 ml intramuscular dose of the bacterin, and the 18 control heifers received a 2 ml intramuscular dose of a sterile saline placebo.

All heifers in both treatment groups were then challenged with two virulent cultures of *Escherichia coli*. Using strains B117 and B44, 40,000 to 70,000 bacteria were infused into the teat of one rear quarter of each udder at 8-38 days post-parturition, which was 27-122 days after vaccination with the bacterin or placebo.

In the 24-hour period following bacterial challenge, each heifer was observed at two-hour intervals, and the following data were collected from each heifer:

1) body temperature (°F)
2) pulse rate
3) respiratory rate
4) number of *E. coli* colonies re-isolated from quarters at 4, 8,12 and 24 hours post-challenge
5) number of rumen contractions, and
6) somatic cell counts (SCC) on milk samples at 0, 12 and 24 hours.

Also, the following subjective observations of the level of mastitis were made; each parameter was rated based on a scale from zero to three, with zero used as normal:

7) inflammatory color of udder quarter infused
8) hardness of infused quarter
9) degree of udder swelling or increase in size, and
10) appearance of milk (degree of watery discoloration and presence of clots).

The following systemic effects were also noted, and each was rated using the same zero to three scale with zero as normal:

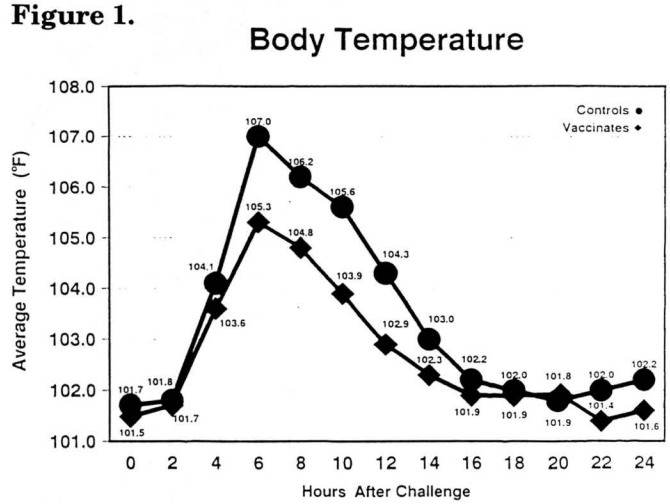
11) degree of anorexia
12) degree of lethargy
13) degree of dehydration, and
14) degree of looseness of feces.

Data collected were averaged within each treatment group and compared at each interval of observation.

Results

Average body temperature of heifers in both treatment groups started rising within two hours of the challenge, and peaked at six hours post-challenge (Figure 1). The control group had consistently higher body temperatures than the vaccinates, and the control average peaked at 107.0°F while the vaccinates peaked at 105.3°F. Average pulse and respiratory rates (Figures 2 and 3, respectively) followed a similar pattern, as values for the control group were consistently higher although the difference in respiratory rates was not significant.

Figure 1.



*bovine Pili Shield™, Grand Laboratories, Inc., Larchwood, Iowa 51241, U.S. Vet License No. 303

Figure 4 shows the average number of *E. coli* colonies re-isolated from challenged udder quarters at 4, 8, 12 and 24 hours post-challenge. On average, less than seven colonies could be cultured from the vaccinated heifers while over 100 (too numerous to count) were cultured from many of the control heifers. Average number of rumen contractions (Figure 5) was similar between the two treatment groups. Figures 6 and 7 show changes in somatic cell counts (SCC) of challenged and unchallenged quarters for periods of 7-14 days pre-challenge, at time of challenge, and 12 and 24 hours post-challenge. The differences between treatment groups were not significant in either figure, but significant increases in SCC occurred in both infected and non-infected quarters in both groups post-challenge.

Figure 2.

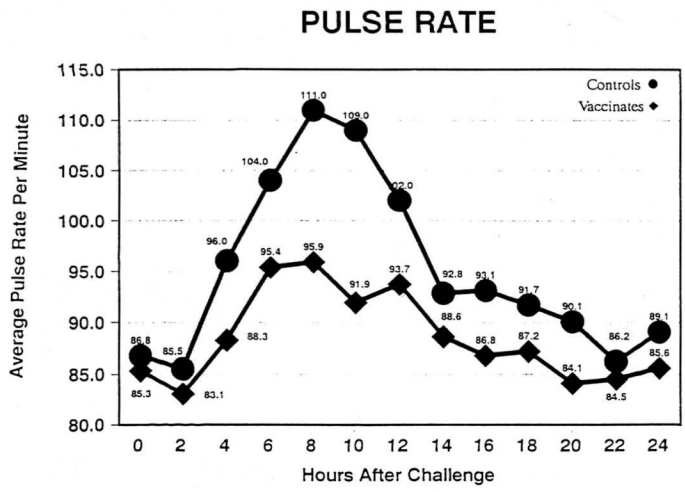


Figure 3.

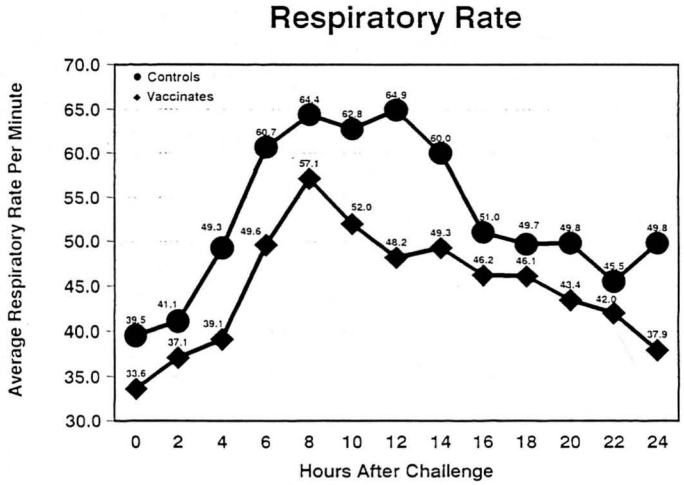


Figure 4.

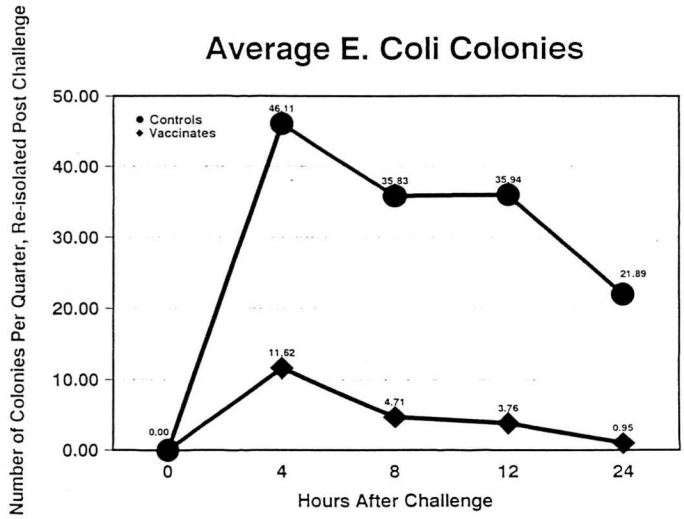
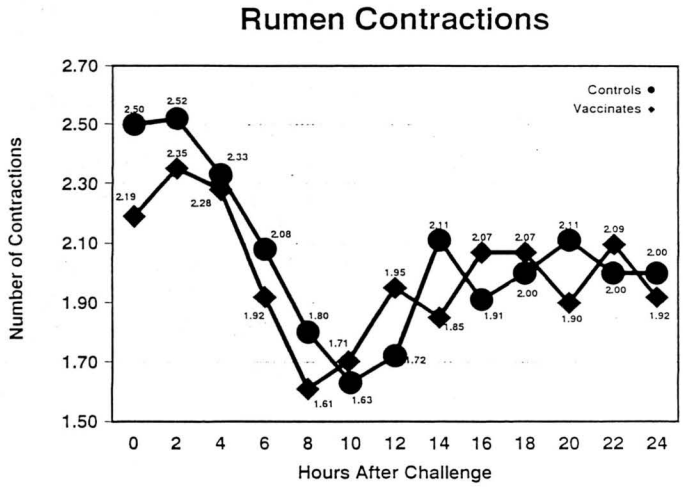


Figure 5.



Average degree of change in inflammatory color of infused quarters (Figure 8), average degree of quarter hardness (Figure 9) and average degree of udder swelling (Figure 10) were all consistently greater in the control animals. Although appearing similar up to eight hours post-challenge, afterwards the milk obtained from the control heifers appeared more watery and had more clots than did the milk from the vaccinated heifers (Figure 11).

Both the degree of anorexia (Figure 12) and degree of lethargy (Figure 13) were greater in the control group after six hours post-challenge. While one heifer in the vaccinated group showed slight dehydration, average dehydration in the vaccinated group was negligible (Figure 14), and only slightly higher in the control group. Average consistency of feces (Figure 15) was erratic, and did not show a difference between treatment groups.

Figure 6.

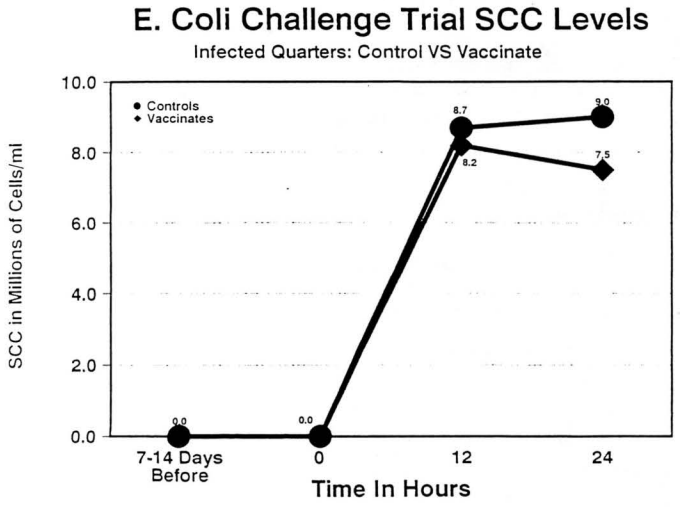


Figure 7.

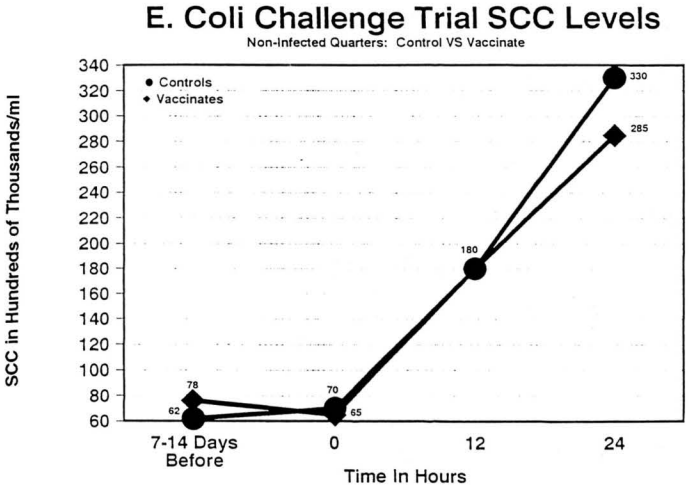


Figure 8.

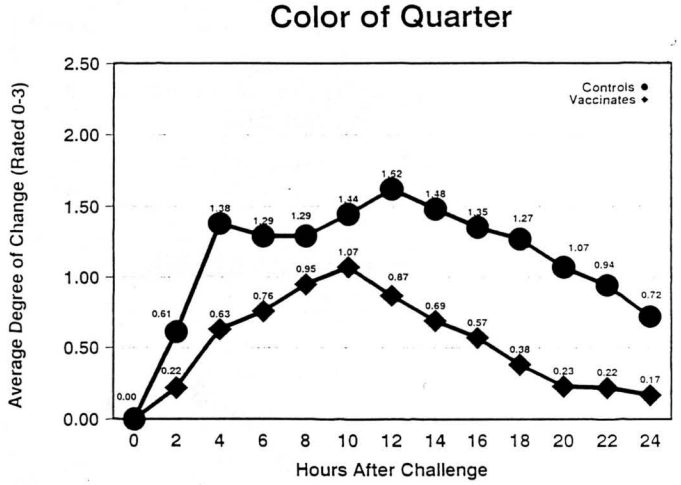


Figure 9.

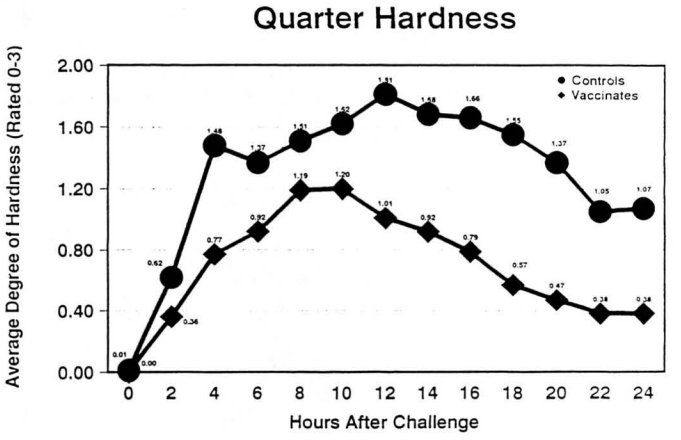


Figure 10.

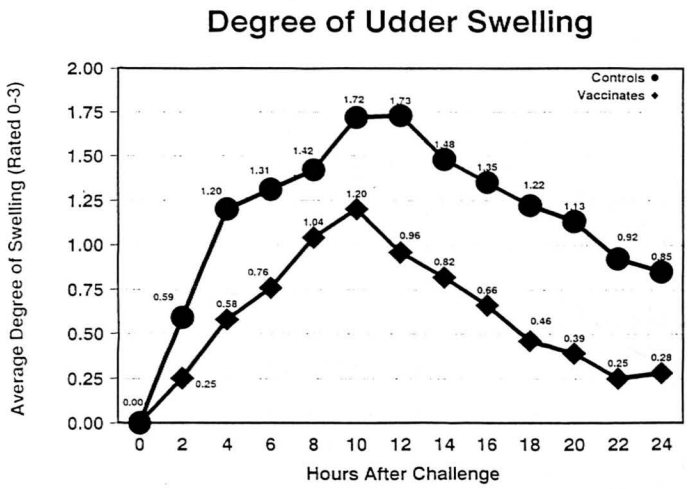


Figure 11.

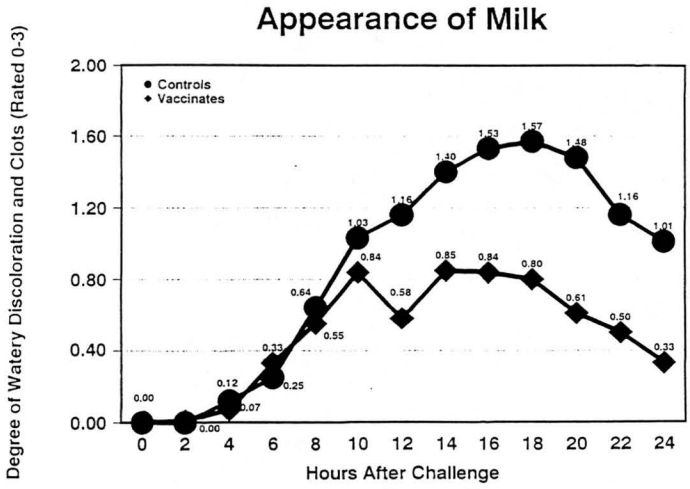


Figure 12.

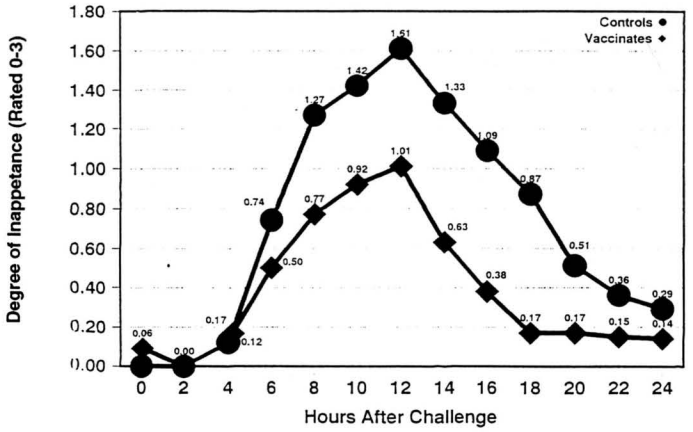


Figure 13.

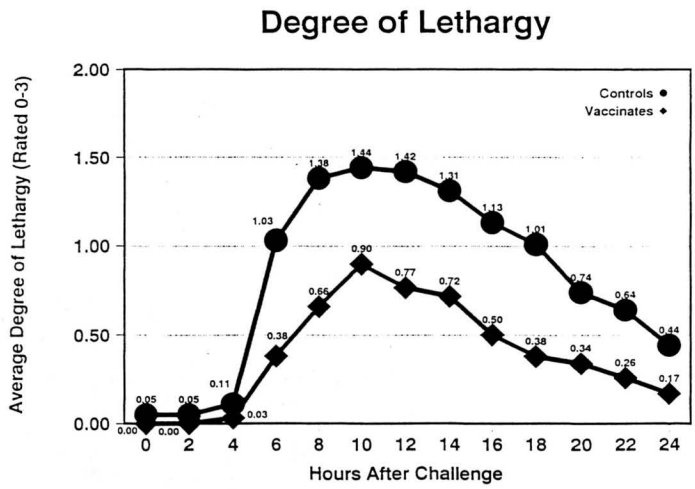


Figure 14.

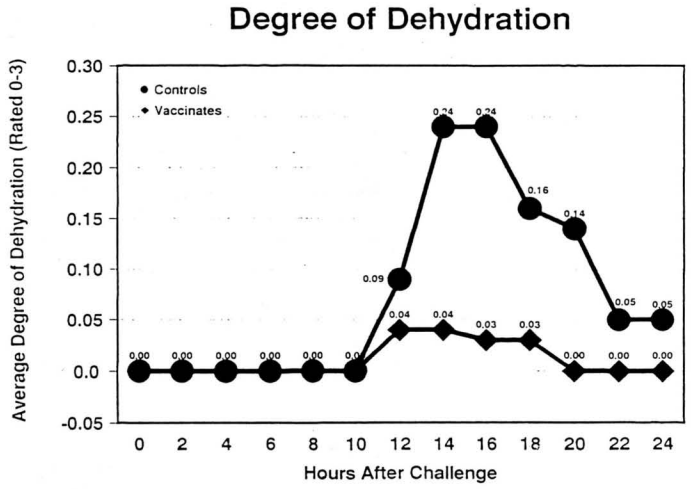
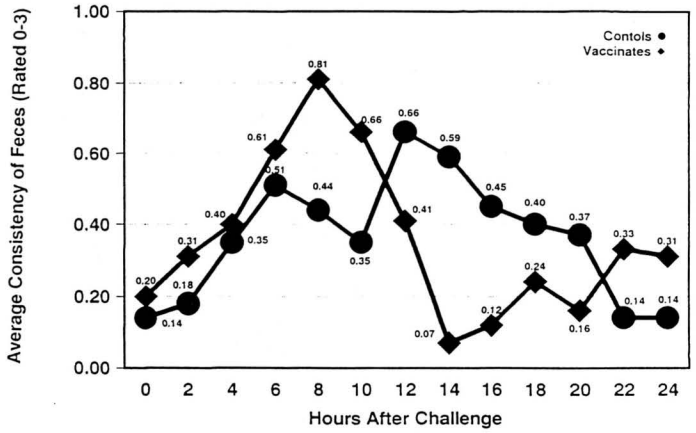


Figure 15.



Discussion

In 1982, the National Mastitis Council estimated that mastitis in beef and dairy cattle had cost producers in the United States over $2 billion annually each of the previous 10 years.[15] As mentioned earlier, while routine mastitis control measures are used to successfully reduce subclinical mastitis caused by the gram-positive pathogens, they do not reduce the incidence or significance of coliform organisms.[2-6] However, newer procedures such as pre-dipping and proper drying of teats pre-milking, do help reduce the incidence of coliform mastitis. An effective vaccine could add to protection against coliform mastitis.

In this challenge trial, results indicate that a single dose of Pili Shield™ can reduce the severity of clinical coliform mastitis in a dairy herd. Eleven of the 14 parameters used to monitor the level of mastitis showed the beneficial effects of the bacterin; the only parameters that did not show significant differences were the number of rumen contractions, consistency of feces and somatic cell count.

Another investigator[17] found that pre- and postpartum injections of a mutant (strain J5) *E. coli* vaccine reduced the rate of clinical coliform mastitis from 12.8% to 2.6%. Other field studies[18] have concurred that administration of bovine Pili Shield™ may reduce the incidence of clinical coliform mastitis.

References

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Studies on virulence factors of *Escherichia Coli* isolated from cows with acute mastitis.

C. Valente, P. Cardaras, A. Ciorba, and B. Tesei.
Arch. Vet. Ital. (1988) 39, 254-260.

Escherichia coli isolated from udders with acute mastitis were identified biochemically and serologically. The bacteria were then tested for mannose haemagglutination, enterotoxin production, serum resistance and invasiveness, characteristics normally associated with *E. coli* isolated from calves with enteric syndromes. *E. coli* from the udder were a serologically heterogeneous group without the characteristics that are usually correlated with *E. coli* of intestinal origin.