Pfizer Animal Health
Technical Bulletin

February 2012

ENVIRACOR™ J-5 aids in the control of clinical signs associated with *Escherichia coli (E. coli)* mastitis

Summary

The challenge data presented in this technical bulletin was completed as part of a master seed requalification program conducted by Pfizer Animal Health.[1] The trial was designed to evaluate the physiological and microbiological changes and the duration of mastitis in cows challenged with *Escherichia coli* (*E. coli*), which were either vaccinated with ENVIRACOR™ J-5 or a placebo.

- The duration of *E. coli* mastitis was 64 hours shorter in cows vaccinated with ENVIRACOR J-5 compared with placebo-vaccinated cows ($P \leq 0.05$).
- Vaccinates had significantly lower *E. coli* viable counts for study periods 0 to 24 hours and 0 to 21 days post-challenge than placebo-vaccinated cows ($P \leq 0.05$).
- Vaccinated cows experienced a significant reduction of *E. coli* shedding by approximately half compared with placebo-vaccinated control cows ($P \leq 0.04$).
- Vaccinated cows experienced 13 times higher milk antibody titers prior to challenge compared with placebo-vaccinated control cows ($P \leq 0.05$).

Introduction

Clinical mastitis due to environmental pathogens can be detrimental to dairy operations, causing fever, abnormal milk, lack of appetite, excessive udder edema, diarrhea, dehydration, dramatic drops in milk products and, in severe cases, death. It has been estimated that on average, clinical mastitis could cost as much as $378 per case, based on disease costs, including drug expenses for treatment, discarded milk, labor costs and losses attributable to premature culling.[2] When considering approaches to managing mastitis on the farm, a systems approach, including management practices, proper milking procedures and nutrition in addition to vaccination programs should be emphasized.

While vaccination is not a substitute for proper management of mastitis, it should be a component of an overall plan to help reduce the risk of disease. Though there are several *E. coli* mastitis vaccines available, differences exist that should be considered when selecting

This trial used a challenge model developed by Pfizer Animal Health to demonstrate ENVIRACOR™ J-5 efficacy.

a vaccine for disease management. Extensive research has helped document the efficacy and safety of ENVIRACOR™ J-5 in cattle. The initial research performed by Dr. James S. Cullor at the University of California-Davis introduced ENVIRACOR J-5 as a new tool to combat mastitis with a favorable efficacy (72 percent less incidence of clinical coliform mastitis and less than 2 percent of affected cows having a repeat event) and safety profile (free endotoxin activity below 200 nanograms per dose with minimum adverse reactions in calves or pregnant cows).[3] Cullor's research validated previous efforts by Tyler, et al., that had identified ENVIRACOR J-5 as an effective vaccine based on its partially shared antigenic structure with Gram-negative mastitis pathogens, its ability to function as an immunogen inducing a humoral response, and the ability to help prevent infection and/or reduce the severity of clinical disease.[4] The safety profile of ENVIRACOR J-5 also has been confirmed in research via comparison of endotoxin units to other commercially available vaccines.[5,6]

The use of ENVIRACOR J-5 has been a valuable component of mastitis control vaccination programs on dairy operations since its introduction in 1993. This technical bulletin outlines a mastitis challenge model that was used to evaluate the efficacy of an *E. coli* mastitis vaccine. Results are consistent with previous research, demonstrating higher specific serum antibody titers, shortened duration of *E. coli* mastitis infection and reduced shedding of *E. coli* (all, $P \leq 0.05$).

Vaccinated and control cows were challenged with a heterologous strain of *E. coli* 21 +/- 7 days after calving.

Materials and methods

The study design involved healthy Holstein or Holstein-cross cows in their second, third or fourth lactation that were confirmed to be 193 ± 7 days pregnant and had four healthy quarters. Cows also were required to have a somatic cell count (SCC) ≤200,000, have negative quarter culture results and be serologically negative for bovine leukemia virus (BLV) or, if positive, have lymphocyte counts of <10,000/µL. Cows were randomly assigned to two groups, with group T01-Control cows receiving saline (placebo) injections and group T02 cows vaccinated with ENVIRACOR J-5, per label directions. The 5.0 mL doses of placebo or bacterin were administered subcutaneously on alternating sides of the neck.

Challenge occurred at 21 ± 7 days after calving. Cows were moved from a commercial dairy to the study facility 12 days before challenge to allow for acclimation. They were milked twice daily from arrival until the end of the challenge phase. On the day of challenge, after evening milking, 55 cows were challenged with a heterologous strain of *E. coli* in the left-front quarter. Forty-five cows met the inclusion criteria and were retained in the final analysis data set (25 in T01-Control group and 20 in T02-ENVIRACOR J-5 group). Efficacy was evaluated based on clinical signs and quantitative milk culture and quality variables associated with mastitis (see Table 1). Udder structure assessed the presence or absence of mammary disease evidenced by heat, pain or redness. Milk bovine serum albumin (BSA) served as an internal measure of vascular or cellular damage consistent with mastitis. Onset of clinical *E. coli* mastitis was defined as at least one abnormal udder structure and one abnormal milk quality variable and a positive *E. coli* milk culture in the challenged quarter. The cow was considered to have continuing *E. coli* mastitis if she had at least one abnormal udder structure and one abnormal milk quality, and/or a positive *E. coli* milk culture in the challenged quarter.

Clinical signs associated with mastitis (rectal temperature, attitude, milk appearance and udder evaluation) were observed and recorded for all cows at 3, 6, 9, 12 and 23 hours (± 15 minutes) and then twice daily, at each milking on Days 2 to 21 post-challenge. Total milk yield, total milk conductivity and cow activity also were monitored at each milking. In addition to clinical observations, milk samples were collected at 3, 6, 9, 12, 15, 18, 21 and 23 hours (± 15 minutes) and then twice daily through 14 days post-challenge, and daily during morning

Table 1. Variables that defined clinical mastitis

Variable	Normal	Abnormal
Milk conductivity	<15% increase from baseline	≥15% increase from baseline
Udder evaluation score	0	≥1
Milk appearance score	0	≥1
Milk somatic cell count*	<200,000 cells/mL	≥200,000 cells/mL
Milk bovine serum albumin**	<0.30 mg/mL	≥0.30 mg/mL
Challenge quarter culture status for *E. coli*	Negative	Positive

*Somatic cell count ranges derived from the National Mastitis Council, *Udder Topics*, December 1997, and from the *Escherichia coli* Mastitis Seminar, Nov. 15, 2007, Richland, Mich.
**Milk bovine serum albumin values derived from Pfizer Animal Health study reports and technical notes and concentrations of glucocorticoids, bovine serum albumin, and somatic cells in mastitic milk. *J Dairy Sci* 1981;64(11):2258-2261.

milking only on Days 15 through 21 post-challenge. These laboratory samples were used to perform quantitative *E. coli* culture, milk BSA and SCC assays. Blood samples for serum also were collected 3 and 7 days post-challenge to determine ENVIRACOR J-5 specific antibody titers. To confirm cows continued to meet inclusion criteria, quarter milk samples were collected from each cow at 14 and 21 days post-challenge for microbiological evaluation and a composite of the three nonchallenged quarters for SCC determinations. Blood samples also were collected from each cow at 21 days post-challenge to screen for BLV titers and lymphocyte count to confirm continued inclusion in the study.

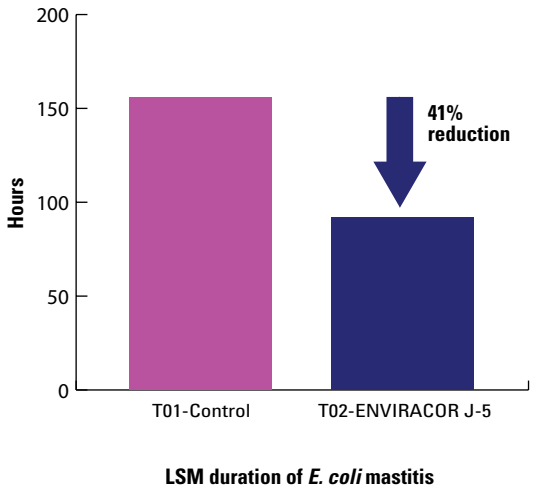
Data summaries and analyses were performed by using a centralized data management system (SAS/STAT, version 9.1 or higher, SAS Institute, Cary, N.C.). Data were analyzed by using a mixed model or a categorical procedure, with the cow as the experimental unit and a 5 percent level of significance ($P \leq 0.05$). The control claim was based on two related but distinct clinical progressions following challenge: 1) severity or magnitude of physiological changes resulting from the challenge and 2) the duration of mastitis (from challenge until 21 days post-challenge).

Results

Eleven cows calved later and nine calved earlier than allowed for inclusion in the challenge phase of the study. Five cows were removed after BLV testing and eight for mastitis unrelated to the challenge. Six cows were excluded for other health-related reasons not associated with vaccination or challenge. More than 70 percent of the T01-Control group cows had *E. coli* mastitis with onset occurring through 24 hours post-challenge, so the challenge model was considered valid. All included cows (100 percent) developed *E. coli* mastitis (see Table 2). There were no signficant adverse events except for some mild injection-

Duration of *E. coli* mastitis was 64 hours shorter (more than 2.5 days) in vaccinated cows. ($P \leq 0.05$)

Figure 1. Duration of *E. coli* mastitis



LSM duration of *E. coli* mastitis

Table 2. Onset and duration of *E. coli* mastitis

Treatment	Animals with *E. coli* mastitis, %	Time to positive culture, hours	Onset of *E. coli* mastitis, hours	LSM duration of *E. coli* mastitis, hours (95% CI)
T01-Control (n=25)	100	3-6	9-15	156.02[a] (97.30-214.74)
T02-ENVIRACOR J-5 (n=20)	100	3-6	6-15	92.00[b] (72.17-111.83)

[a,b] Values with different superscripts within each column are significantly different ($P \leq 0.05$).

site swellings that resolved before the next treatment and were consistent with bacterin administration.

Abnormal challenge quarter milk appearance and clinical status were tracked and reported in regard to the onset and duration of *E. coli* mastitis in both groups. Twenty-four of 25 T01-Control cows and 19 of 20 T02-ENVIRACOR™ J-5 cows had milk samples positive for *E. coli* at 3 hours post-challenge; the remaining cows, one each in the T01-Control and T02-ENVIRACOR J-5 groups, were positive at 6 hours post-challenge. The onset of *E. coli* mastitis occurred at 9 hours post-challenge for eight of 25 cows, at 12 hours for 16 cows, and 15 hours for one cow in the T01-Control group. The onset of *E. coli* mastitis occurred at 6 hours post-challenge for one cow, at 9 hours for six cows, at 12 hours for 11 animals, and at 15 hours for two cows in the T02-ENVIRACOR J-5 group. Duration of *E. coli* mastitis for the acute phase (0 to 24 hours post-challenge) was 16.84 hours for T01-Control cows and 16.90 for T02-ENVIRACOR J-5 cows and was not significantly different (P=0.92). Duration of *E. coli* mastitis for Days 0 to 21 post-challenge was 156.02 hours for T01-Control cows and 92 hours for T02-ENVIRACOR J-5 cows and was significantly different. Thus, the duration of *E. coli* mastitis was 41 percent shorter in vaccinated cows. These results are consistent with previous trials that have demonstrated shorter duration of intramammary *E. coli* infection for cows vaccinated with ENVIRACOR J-5.[7,8] The

stratified mitigated fraction estimate for duration was 19.9 and the 95 percent CI was 12.7 to 31.3. The lower 95 percent CI was above zero, confirming that the duration of *E. coli* mastitis was mitigated in vaccinates compared with controls.

There was no significant overall treatment effect for milk BSA, SCC or conductivity, so no comparisons were made of these variables (see Table 3). However, the overall mean challenged quarter *E. coli* milk viable count was significantly lower ($P \leq 0.05$) in vaccinated cows compared with controls (see Figure 2, Page 6). Mean milk *E. coli* viable count results (CFU/mL) for 0 to 24 hours, 2 to 7 days, 8 to 21 days and 0 to 21 days were 2,426 for T01-Control and 1,104 for T02-ENVIRACOR J-5, 5 for T01-Control and 3 for T02-ENVIRACOR J-5, 0 for T01-Control and 0 for T02-ENVIRACOR J-5, and 8 for T01-Control and 5 for T02-ENVIRACOR J-5, respectively. Vaccinates had significantly lower *E. coli* culture counts ($P \leq 0.05$) for study periods 0 to 24 hours and 0 to 21 days post-challenge.

Antibody titers in serum and milk appear in Figure 3 and Figure 4 (Page 6). Overall mean serum and milk total IgG whole cell ELISA antibody titers were significantly higher ($P \leq 0.05$) in vaccinated cows compared with controls. Mean serum total IgG whole cell ELISA antibody titers results for the first milk post-calving were 14,486.4 for the control group and 197,982.1 for vaccinates.

Discussion

The challenge was effective, eliciting *E. coli* mastitis in all cows. However, the ENVIRACOR J-5 bacterin stimulated significantly higher total IgG antibody titers in milk and serum in vaccinated vs. placebo-vaccinated cows ($P \leq 0.05$). Clinical progression in *E. coli* mastitis and the duration of mastitis were both reduced in vaccinates compared with controls. Vaccinated

Table 3. Udder and milk variables in challenged quarters, least squares means (95% confidence intervals)

Treatment	0 to 24 Hours	2 to 7 Days	8 to 21 Days	0 to 21 Days
Milk bovine serum albumin (mg/mL)				
T01: Control	0.98 (0.92-1.04)	0.42 (0.37-0.47)	0.09 (0.05-0.14)	0.36 (0.32-0.40)
T02: ENVIRACOR J-5	1.01 (0.93-1.09)	0.45 (0.39-0.51)	0.10 (0.05-0.15)	0.38 (0.34-0.43)
Somatic cell count (cells/mL)				
T01: Control	847,473 (647,669-1,108,915)	2,663,626 (2,097,153-3,383,112)	180,247 (143,584-226,273)	536,247 (429,117-670,122)
T02: ENVIRACOR J-5	1,115,922 (853,428-1,459,154)	3,143,565 (2,444,336-4,042,817)	184,521 (144,761-235,202)	601,111 (473,528-763,069)
Milk conductivity (mmHO)				
T01: Control	11.1 (10.7-11.5)	9.9 (9.6-10.1)	9.4 (9.2-9.7)	9.6 (9.4-9.9)
T02: ENVIRACOR J-5	10.8 (10.3-11.2)	9.6 (9.4-9.9)	9.2 (9.0-9.5)	9.4 (9.2-9.7)

Figure 2. Challenge quarter *E. coli* viable counts 24 hours post-challenge (CFU/mL)

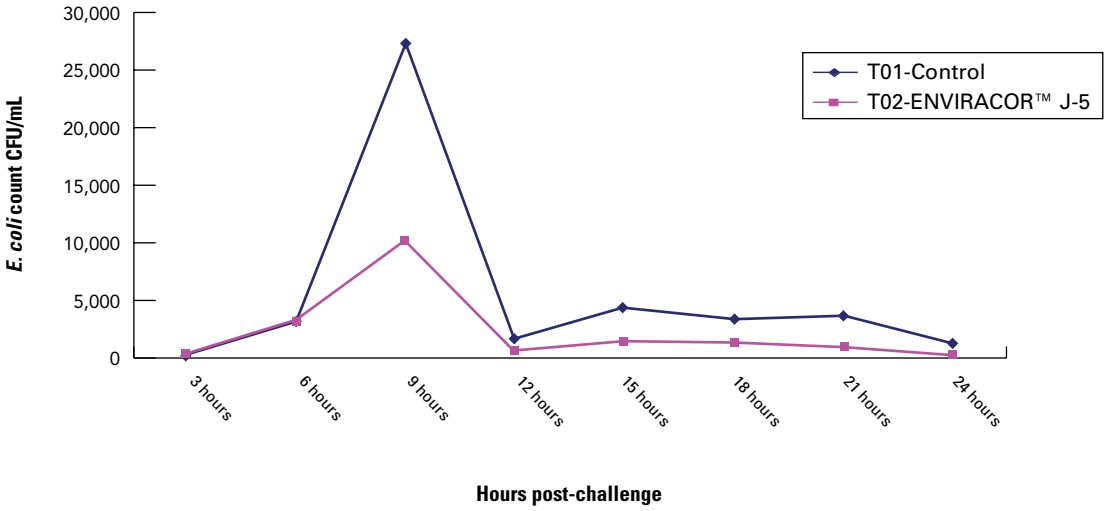


Figure 3. Antibody titers in serum-ELISA IgG titers

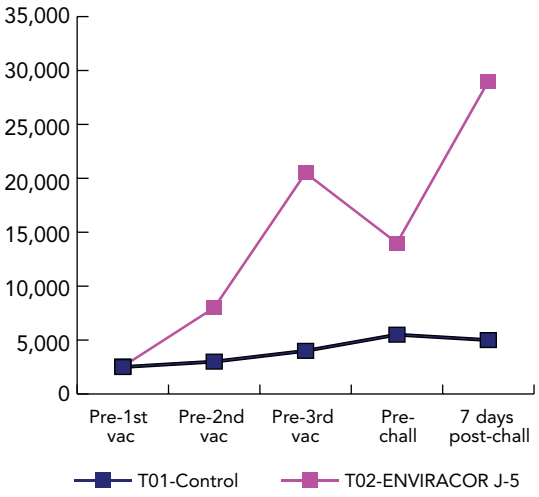
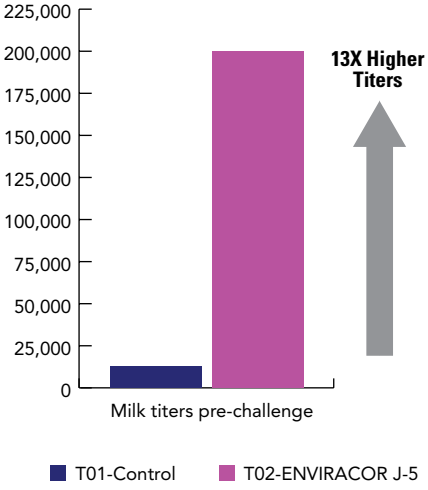


Figure 4. Antibody titers in milk



cows resumed normal production 64 hours earlier than control cows, and the return to production can be expected to translate into more salable milk. Vaccinated cows also shed less potentially infective *E. coli* bacteria than their placebo-vaccinated counterparts, which could help in control of this environmental pathogen.

These new data built on more than 20 years of experience with ENVIRACOR J-5.

Conclusions

ENVIRACOR J-5 was introduced in 1993 and is supported by an extensive body of research that has helped document the safety and efficacy for use in cattle. The challenge study discussed in this technical bulletin represents an *E. coli* mastitis challenge model utilized to evaluate the efficacy of an *E. coli* bacterin and was designed to evaluate the physiological changes and the duration of mastitis in cows vaccinated with ENVIRACOR J-5 compared with placebo-vaccinated cows. Results demonstrate that *E. coli*-vaccinated cows have a shorter duration of *E. coli* mastitis, lower *E. coli* viable counts and higher antibody titers in milk and serum compared with placebo-vaccinated cows. The challenge data presented in this technical bulletin supports the use of ENVIRACOR J-5 vaccine as an integral component of an overall mastitis management program.

Vaccinated cows shed significantly less ($P \leq 0.05$) *E. coli* into the environment during the 3 weeks post-challenge than placebo-vaccinated cows.

Differences exist and must be considered by producers or veterinarians using or recommending an *E. coli* bacterin.

References

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TAKE TIME
OBSERVE LABEL DIRECTIONS

Pfizer Animal Health