J Vet Intern Med 1999;13:36–39

Effect of Passive Transfer Status and Vaccination with *Escherichia coli* (J5) on Mortality in Comingled Dairy Calves

Jeff W. Tyler, Dale D. Hancock, Leilani Wilson, Fred Muller, Denise Krytenberg, and Susan Bradish

The effect of vaccination with a commercially available R-mutant coliform mastitis vaccine on the survival of comingled dairy calves on a farm with endemic salmonellosis was examined. A total of 864 calves were randomly assigned to either vaccine (n = 435) or control (n = 429) groups. Passive transfer status of each calf was determined using refractometer determination of serum total protein concentration. Logistic models were developed to determine the effects of vaccine group and passive transfer status on calf survival to 100 days of age. In a model in which serum protein concentration was treated as a categorical variable, increasing serum total protein concentrations were associated with decreased mortality until these concentrations exceeded 6.0 g/dL. Calves with serum protein concentrations >6.0 g/dL had increased risk for mortality compared with calves with serum protein concentrations >5.5 g/dL but ≤6.0 g/dL. This increased risk for mortality was supported by the results of a logistic model in which serum protein concentration was treated as a continuous variable. The increased risks associated with high serum protein concentration probably reflect the effect of dehydration in calves with occult disease. Neither model demonstrated any significant association between vaccination status and survival to 100 days of age. Based on these results, the routine immunization of calves cannot be recommended as a strategy to prevent mortality on farms with endemic salmonellosis.

Key words: Failure of passive transfer; *Salmonella dublin*; Salmonellosis; Vaccines.

Although somatic antigens vary greatly among gram-negative bacteria, portions of gram-negative lipopolysaccharide have structural and antigenic homology.[1,2] This homologous core antigen structure makes the rough mutant bacteria (R mutant), such as *Escherichia coli* (J5), logical candidates for broad spectrum vaccine antigens.[1–3] *Escherichia coli* J5 is an R_c mutant that lacks the enzyme udg galactose epimerase, required for complete somatic side chain assembly. Incomplete side chain assembly exposes homologous core antigens, which include lipid A and KDO. These structures are highly conserved and cross-reactive among unrelated gram-negative bacteria.[4,5] The basis of R mutant immunization is the hypothesis that vaccination with homologous core antigens may provide protection against disease caused by unrelated gram-negative bacteria. Examples of diseases for which heterologous protection using *E. coli* J5 as a vaccine antigen has been demonstrated include *Actinobacillus pleuropneumoniae* infections of pigs,[6,7] gram-negative mastitis of dairy cows,[8–10] and *Edwardsiella ictaluri* septicemia in channel catfish.[11]

Although Cullor et al[12] demonstrated that calves vaccinated with an experimental bacterin containing *E. coli* J5 were protected against virulent challenge with *Salmonella dublin*, field trials with similar vaccines have had mixed results. Daigneault et al[13] demonstrated that calves vaccinated with *E. coli* J5 that were reared on a farm with a high level of general health and management had significantly decreased mortality, whereas vaccinated calves residing on a farm with less optimal calf health had significantly increased mortality. More recently, Selim et al[14] observed that calves vaccinated with *E. coli* J5 did not have altered mortality rates when compared with control calves.

Several R mutant bacterins are marketed for use in cattle in the United States. Label claims are restricted to the prevention of coliform mastitis or endotoxemia. Although none of these products are specifically labeled for the prevention of salmonellosis or nonspecific mortality in neonatal calves, the presence of shared or homologous core antigen seems to support the use of R mutant bacterins in the prevention of these diseases. Presently, no study has included a critical examination of the efficacy of commercially available R mutant bacterins for these alternative uses. In the present study, we examined the efficacy of an R mutant bacterin in the prevention of mortality in calves on a farm with endemic salmonellosis.

Materials and Methods

Study Site

A calf-rearing farm located in central Washington state was selected as a study site. This farm rears 3,000 calves/year. On a weekly basis, the owner of the facility obtained 30–60 calves from approximately 20 client dairies and transported these calves as a comingled group to the calf-rearing facility. Calves were placed in individual calf hutches, fed 2 L milk replacer twice daily, and offered hay, concentrate, and water ad libitum. Calves were weaned at 8 weeks of age and housed in groups thereafter.

Calves on this farm are periodically monitored for passive transfer of immunoglobulin using refractometry for total serum protein concentration. In the year prior to the study inception, approximately 30% of calves presented to the study site had serum protein concentrations <5.0 g/dL and consequently were deemed to have at least partial failure of passive transfer (FPT).[15]

The primary neonatal health problem in this herd appeared to be the result of *S. dublin* infections. Clinical manifestations of disease have included diarrhea, acute mortality, respiratory disease, and apparent septic physitis. Endemic salmonellosis on this farm had been confirmed by fecal cultures from acutely ill calves and by organ cultures from calves that succumbed to clinical disease. These samples were collected and processed before, during, and after the described study.

The large numbers of calves at risk made this farm an ideal setting to assess the efficacy of an R mutant bacterin in the prevention of bovine neonatal salmonellosis. The sample size for this study and

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA. Dr. Tyler is presently affiliated with the Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO.

Reprint requests: Jeff W. Tyler, DVM, PhD, Veterinary Clinical Sciences, University of Missouri, 329 East Campus Drive, Columbia, MO 65211; e-mail: TylerJ@missouri.edu.

Accepted January 5, 1998.

0891-6640/99/1301-0005/$3.00/0

Table 1. Groups defined by serum protein concentrations in calves that either died or survived 100 days.

Serum Protein Concentration (g/dL)	Mortalities	Survivors	Total	Crude Odds Ratio
<4.6	12	157	169	10.32
4.6–5.0	14	241	255	7.84
5.1–5.5	7	214	221	4.42
5.6–6.0	1	135	136	1[a]
>6.0	3	80	83	5.06
Total	37	827	864	

[a] This group was defined as having the baseline risk for mortality, and the odds ratios for other groups were calculated relative to this group.

hence the duration of subject enrollment was based on a 10% baseline mortality, a 50% reduction in mortality attributable to vaccination, a power of 0.80, and a *P* value to reject the null hypothesis of .05.

Sample Collection and Processing

A total of 864 calves were enrolled in the study. Three days after arrival, blood was obtained from the jugular vein of each calf. At the time of enrollment, calves ranged in age from 3 to 10 days. Samples were transported at 4°C to the laboratory and maintained at refrigeration temperatures thereafter. Serum was collected within 18 hours of sample collection after centrifugation. Passive transfer status of calves studied was determined by measurement of total serum protein concentrations using a temperature-compensating refractometer (TS Meter, American Optical, Buffalo, NY). Refractometer determinations of total protein concentrations have been previously determined to correlate closely with serum IgG_1 concentrations, with reported r^2 ranging from .65 to .76 (Parish et al, unpublished data).[15]

Vaccination

All calves entering the facility were identified using uniquely numbered eartags. Calves were randomly assigned to vaccine or control groups using the results of a coin toss. Vaccinated calves received a commercially available coliform mastitis vaccine (*E. coli* bacteria, Upjohn Co, Kalamazoo, MI) at the time of initial enrollment and a second immunization with the same vaccine 2 weeks later. The ability of calves to mount humoral immune responses immunization with *E. coli* J5 in an oil-based adjuvant has been demonstrated in previous studies.[16]

Data Collection

Farm personnel recorded all deaths in individual calf health records on a daily basis. Farm personnel were blinded with regard to each calf's group assignment. Mortality records were collected weekly in conjunction with scheduled farm visits.

Data Analysis

Mean serum protein concentration and its standard error were calculated for the study population. The frequency of calves in strata defined by total serum protein concentration (TSP), mortality, and vaccination status were tabulated using contingency tables. These tables were not analyzed because it was anticipated that subsequent multivariate analyses would remove any confounding effects of passive transfer status on the effect of vaccination status on survival and, consequently, were deemed more trustworthy. The proportion of calves that died in the first 100 days in residence in the study herd was calculated.

Table 2. Mortality in calves classified on the basis of vaccination status with regard to *Escherichia coli* J5 in a study on the effect of vaccination with *E. coli* J5 on mortality in calves.

Vaccination Status	Mortalities	Survivors	Total
Controls	16	413	429
Vaccinates	21	414	435
Total	37	827	864

Stepwise logistic regression was used to develop a model predicting survival to 100 days in the study herd. Independent variables considered included vaccination group, TSP, and the interaction of the 2 terms.[17] Main effects were permitted to enter the model at $P < .10$. At each step the variable with the smallest *P* value was permitted to enter the model. Interactions were considered for inclusion when their component terms were previously included in the model at $P < .10$. Separate models were constructed in which TSP was treated as either a continuous variable or a categorical variable. The categorical model used the following upper limits for 4 classes: 4.5 g/dL, 5.0 g/dL, 5.5 g/dL, and 6.0 g/dL. Using regression equations developed in previous studies, these serum protein concentrations were roughly equivalent to 440, 890, 1,340, and 1,790 mg/dL IgG_1.[15] The model that considered TSP as a continuous variable also considered TSP^2 as an independent variable. Calculations were performed using a statistical software package (BMDP Statistical Software, Los Angeles, CA). Models predicting sick days and treatment and rearing costs were not developed because variability in diagnostic and therapeutic regimens precluded meaningful analysis of these data.

Results

The mean (± SEM) TSP in the study population was 5.14 (± 0.02) g/dL. A total of 424 calves (49%) had TSP ≤ 5.0 g/dL. Of the 862 calves enrolled in the study, 37 (4.3%) died in the first 100 days in residence in the study herd. Observed mortality rates were highest in the 169 calves with ≤ 4.5 g/dL TSP (7.1%) and lowest in the 136 calves with TSP > 5.5 g/dL and ≤ 6.0 g/dL (0.7%) (Table 1). Calves with TSP > 6.0 g/dL had a higher mortality rate (3.6%). Of the 429 calves enrolled as controls, 16 (3.7%) died in the first 100 days after enrollment in the study. Of the 435 calves enrolled as vaccinates, 21 (4.8%) died in the first 100 days after enrollment in the study (Table 2). Vaccinates were overrepresented in the serum protein concentration strata with the highest mortality and underrepresented in the strata with the lowest mortality (Table 3).

In the logistic model that treated TSP as a categorical variable, increasing TSP values were significantly associated with decreasing risk of mortality until these concentrations exceeded 6.0 g/dL (Table 1). Calves with TSP > 6.0 g/dL had an increased risk for mortality compared with calves with TSP > 5.5 g/dL but ≤ 6.0 g/dL. The improvement chi-square *P* value for the inclusion of TSP in the model was .030, and the overall goodness-of-fit chi-square *P* value for the model was .274. Neither vaccination status nor the interaction of vaccination status and TSP was significantly associated with mortality ($P > .10$).

Table 3. Control and vaccinated calves in groups defined by serum protein concentration in a study on the effect of vaccination with *Escherichia coli* J5 on mortality.

Serum Protein Concentration (g/dL)	Controls	Vaccinates	Total
<4.6	77	92	169
4.6–5.0	127	128	255
5.1–5.5	117	104	221
5.9–6.0	70	66	136
>6.0	38	45	83
Total	37	827	864

The logistic model predicting mortality as a function of TSP, TSP^2, and vaccination status substantiated the results of the preceding analysis. The first dependent variable to enter the model was TSP (improvement χ^2 $P = .046$, cumulative goodness-of-fit χ^2 $P = .720$). The second dependent variable to enter the model was TSP^2 (improvement χ^2 $P = .010$, goodness-of-fit χ^2 $P = .870$). Neither vaccination status nor the interaction between vaccination status and serum protein concentration was significantly associated with mortality ($P > .10$).

Discussion

Immunization with a commercially available R mutant bacterin had no significant effect on mortality. However, the study site may not have been representative of all dairy farms. The calves studied originated from several farms, the farm was endemically infected with *S. dublin*, the proportion of calves with partial or complete FPT was high, and mortality rates were relatively low. Alterations in any of these conditions could have altered either the benefits or risks associated with such an immunization program.

Based on the results of this study, 3 important issues arise that were peripheral to the stated purposes. First, as anticipated, passive transfer status is an important determinant of calf health and survival. Calves with complete, severe FPT (<4.5 g/dL serum protein concentration) were approximately 10 times more likely to die in the first 100 days of life than were calves with >5.5 g/dL and ≤6 g/dL serum protein concentrations. This risk of mortality decreased as serum protein concentrations increased. Calves with serum protein concentrations >5.5 g/dL and ≤6.0 g/dL were least likely to die in the first 100 days of life. It is tempting to set 5.5 g/dL as a goal for serum protein concentration in calves; however, only 249 (28%) of the calves studied had serum protein concentrations exceeding this threshold value. Farm managers may not be able to consistently meet such a goal without dramatic and rigorous efforts at intervention. Some have advocated routine administration of a single 4-L colostrum feeding using an esophageal feeder in the first hours of life.[18] This practice should be strongly considered on dairy farms with high neonatal mortality.

The second issue raised was that calves with serum protein concentrations >6 g/dL were at increased risk for mortality. This increased risk was substantiated by the increased mortality rate observed in the categorical model and the inclusion of a quadratic term in the model that treated serum protein concentration as a continuous variable. These increased risks associated with high serum protein concentration may reflect the effect of dehydration in calves with occult disease. Alternatively, these high serum protein concentrations could reflect the endogenous production of globulins in calves undergoing an active inflammatory process.

Calves can be reared successfully under conditions of endemic salmonellosis and FPT. The relatively low mortality in both controls (3.7%) and vaccinated calves (4.8%) supports this assertion. The design of this study precluded an analysis of the effects of hygiene, nutrition, housing, and treatment strategies on calf survival; however, all of these determinants are probably critical in the survival of comingled calves raised under less than ideal conditions.

Based on the results of this study, we are unable to recommend the use of R mutant bacterins in neonatal calves. Also, it is clear that passive transfer is important in maintaining the health of neonatal calves.

Acknowledgment

We acknowledge the assistance of owners and staff of the Reuble Calf Ranch.

References

1. Elbein AD, Heath EC. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*: Properties of a uridine diphosphate galactose 4-epimeraseless mutant. J Biol Chem 1965;5: 1919–1925.

2. Luderitz O, Staub AM, Westphal OM. Immunochemistry of O and R antigens of *Salmonella* and related Enterobacteriaceae. Bacteriol Rev 1966;30:192–225.

3. Tyler JW, Cullor JS, Speir SJ, et al. Immunity targeting common gram-negative core antigens. J Vet Intern Med 1990;4:17–25.

4. Tyler JW, Spears H, Cullor JS, et al. Antigenic homology among gram-negative organisms isolated from cattle with clinical mastitis. J Dairy Sci 1991;74:1235–1242.

5. Tyler JW, Spears H, Nelson R. Antigenic homology of endotoxin with the coliform mastitis vaccine strain, *Escherichia coli* O111:B4 (J5). J Dairy Sci 1992;75:1821–1825.

6. Fenwick BW, Cullor JS, Osburn BI, et al. Mechanisms involved in protection provided by immunization against core lipopolysaccharides of *Escherichia coli* J5 from lethal *Haemophilus pleuropneumoniae* infections in swine. Infect Immun 1986;53:298–304.

7. Fenwick BW, Cullor JS, Osburn BI, et al. Mortality in swine herds endemically infected with *Haemophilus pleuropneumoniae*: Effect of immunization with cross-reacting lipopolysaccharide core antigens of *Escherichia coli.* Am J Vet Res 1987;47:1888–1891.

8. Gonzales R, Cullor JS, Jasper DE, et al. Prevention of clinical coliform mastitis in dairy cows by a mutant *Escherichia coli* vaccine. Can J Vet Res 1989;53:301–307.

9. Cullor JS. The *Escherichia coli* J5 vaccine: Investigating a new tool to combat coliform mastitis. 1991;86:836–844.

10. Hogan JS, Smith KL, Toddhunter DA, et al. Field trial to determine efficacy of an *Escherichia coli* J5 mastitis vaccine. J Dairy Sci 1992;75:78–84.

11. Tyler JW, Klesius PL. Protection against enteric septicemia of catfish (*Ictalurus punctatus*) by immunization with the R-mutant, *Escherichia coli* (J5). Am J Vet Res 1994;55:1256–1260.

12. Cullor JS, Fenwick BW, Smith BP, et al. Decreased mortality

and severity of infection from salmonellosis in calves immunized with *E. coli* (strain J5) (abstract 352). Proceedings 66th Annual Conference of Research Workers in Animal Disease, Chicago, IL.

13. Daigneault J, Thurmond M, Anderson M, et al. Effect of vaccination with the R mutant, *Escherichia coli* (J5) on morbidity and mortality of dairy calves. Am J Vet Res 1991;52:1492–1496.

14. Selim SA, Cullor JS, Smith BP, et al. The effect of *Escherichia coli* J5 and modified live *Salmonella dublin* vaccines in artificially reared neonatal calves. Vaccine 1995;13:381–390.

15. Tyler JW, Hancock DD, Parish SM, et al. Evaluation of three assays for failure of passive transfer in calves. J Vet Intern Med. 1996; 10:304–307.

16. Tyler JW, Cullor JS, Thurmond MC, et al. Humoral response in neonatal calves following immunization with *Escherichia coli* (strain J5): The effects of adjuvant, age and colostral passive interference. Vet Immunol Immunopathol 1989;23:333–344.

17. Affifi AA, Clark V. Computer-Aided Multivariate Analysis. Belmont, CA: Lifetime Learning.

18. Besser TE, Gay CC. Colostral transfer of immunoglobulins to the calf. Vet Annu 1993;33:53–61.