Comparison of Probiotic and Antibiotic Intramammary Therapy of Cattle With Clinical Mastitis

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Numerous commercial products are available for intramammary infusion of cows with clinical mastitis[1-5]. The majority of these products are antibiotics that require dairy producers to withhold milk from the saleable milk supply for a period of time to avoid antibiotic residues. This loss of saleable milk is an economic concern for producers. Dairy producers must also closely comply with stated withdrawal times to prevent antibiotic residues in the milk.

There has been speculation that the administration of probiotic therapy to infected mammary glands may be an effective treatment of clinical mastitis. It would be economically advantageous if an effective probiotic could be identified that would not require any necessary withdrawal of milk following treatment.

Lactobacillus species, normal microflora of digestive and urogenital tracts, have been reported to have antimicrobial activity in vitro[6] and when administered orally to poultry[7-9] and mice[10] and intravaginally to humans[11,12]. These studies have reported an antagonistic effect of lactobacilli on *Staphylococcus aureus*[9], coliforms[7,8,11,12], and *Pseudomonas aeruginosa.*[10]

The objectives of this study were to compare the effects of treating clinical mastitis with intramammary infusions of either a lactobacillus or an antibiotic product on imtramammary infection cure rate and on milk somatic cell count (SCC).

Materials and Methods

Animals, Housing, and Management

Experimental cattle were a part of the dairy herd of the Ohio State University Agricultural Technical Institute. All cattle were housed in a 100-cow free-stall barn with stalls bedded predominantly with dried sawdust. All manure and wet bedding were removed twice daily and fresh bedding was added weekly. Milking occurred on a 12 hour interval in a double-6 herringbone parlor. Premilking preparation of all teats consisted of forestripping to check for abnormalities, pre-dipping with .25% titratable iodine and removal of soil and residue with individual paper towels at least 30 seconds after predipping. All teats were dipped postmilking with 1.0% titratable iodine. Milk samples were obtained from all lactating cows and analyzed monthly by the Dairy Herd Improvement Association (DHIA) for composite SCC.

Assignment to Treatment Groups

A total of 75 quarters were identified as having clinical mastitis during a 10 month study. Observations of abnormal milk (flakes, clots, or watery secretion) or of an obviously swollen quarter at forestripping was diagnosed as clincial mastitis. Clinical quarters were allotted to one of two groups based upon odd or even cow identification numbers.

Sampling and Treatment Procedures

Quarter foremilk samples were aseptically obtained from all quarters before treatment, at 0 and 12 hours after clinical mastitis was diagnosed (duplicate samples on day 0) and at days 7 to 14 after clinical quarters were treated to determine the microbiological status and SCC. Sampling of mammary quarters, microbiological procedures, and milk SCC procedures were as previously described[13]. All quarter samples were refrigerated and processed within 24 hours of sampling. Quarters were diagnosed as being infected prior to treatment if the same pathogen was isolated from duplicate samples on day 0. Infected quarters were diagnosed as cured if the pathogen isolated on day 0 was not present in both day 7 and 14 samples. Somatic cell counts were expressed as SCC ($\log_{10}$) per ml of milk.

All clinical quarters were aspectically infused postmilking at 12 and 24 hours after being diagnosed.Quarters in the lactobacillus (L) group were infused both times with a commercial probiotic (lacto-bac[a], *Lactobacillus acidophilus* and *Lactobacillus casei,* 90 x 10^6 viable microorganisms), approved for oral use in calves as a microbial supplement, while quarters in the cephapirin (T) group were infused both times with a commercial antibiotic preparation (ToDAY[b], Na cephapirin, 200 mg).

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[a]*Lakeland Vet, Eden Prairie, MN*
[b]*Franklin Laboratories, Amarillo, TX*

Data Analysis

Differences among SCC means were analyzed using the General Linear Model procedure of SAS[14]. Treatment, parity, days in milk, and season were the inital variables included in the model for each analysis. A backwards step-wise elimination procedure was used to determine the final model which included treatment and significant ($P < .05$) independent variables. Analyses used to compare means were Student's t test and analysis of variance. Differences in categorical dependent variables were determined using two-way chi-square analyses[14].

Results

Initial treatment group information is summarized in Table 1. The majority of clinical cases for both treatment groups occurred during the summer months. The average pretreatment SCC of clinical quarters was similar ($P > .05$) for L and T groups. Pathogens were isolated in 57% of the clinical quarters (59% and 57% for L and T, respectively P >.05). Forty-one percent of all clinical quarters and 72% of bacteriologically-positive clinical quarters were infected with major pathogens (*Staphylococcus aureus*, environmental streptococci, or Gram-negative bacilli). There was little difference in total major pathogens between the two groups (38 vs. 44%).

TABLE 1 Summary of parity, days in milk and quarter somatic cell count (SCC) prior to treatment of clinical quarters with lactobacillus (L) and cephapirin (T).

	Treatment group	
	L	T
Total no. of clinical quarters	29	46
Parity ($\bar{X} \pm$ S.D.)	1.3 ± .5	3.0 ± 1.4
Days in milk ($\bar{X} \pm$ S.D.)	178.5 ± 117.3	191.1 ± 143.0
Quarter SCC (log_{10}/ml) ($\bar{X} \pm$ S.D.)	6.3 ± .5	6.2 ± .8

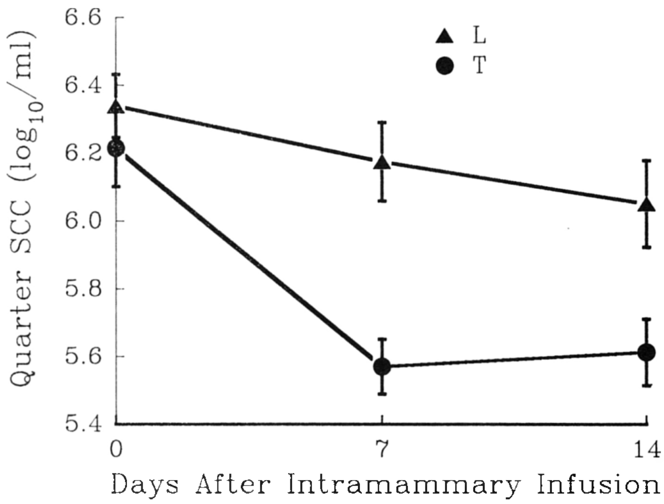
The overall cure rates of infected quarters in the L and T treatment groups were 35.3% and 57.7%, respectively (Table 2 $P = .10$). Cure rates were higher for minor pathogen than for major pathogen infections for both L and T treatments. The greatest difference in cure rate between treatments was found in quarters infected with major pathogens. The main contributor to this difference in cure rate for L and T quarters was the difference in cure rates of Gram-negative bacilli infections. Gram-negative infection cure rates for L and T were 0 and 61.5%, respectively (Table 2, $P = .11$).

The average clinical quarter SCC was higher ($P < .05$) for L than T at 7 and 14 days following therapy (Figure 1). Mean SCC decreased from day 0 to day 7 for T quarters

TABLE 2 Cure rates of clinically-infected quarters treated with lactobacillus (L) or cephapirin (T).

	Treatment group				
	L		T		
Mastitis pathogen	Number Infected	Percent Cured	Number Infected	Percent Cured	P
Staphylococcus aureus	0	---	1	0.0	
Environmental streptococci	7	28.6	6	33.3	
Gram-negative bacilli	4	0.0	13	61.5	
Total major pathogens	11	18.2	20	50.0	.08
Coagulase-negative staphylococci	4	100.0	5	80.0	
Corynebacterium spp	2	0.0	1	100.0	
Total minor pathogens	6	66.7	6	83.3	.39
All pathogens	17	35.3	26	57.7	.10

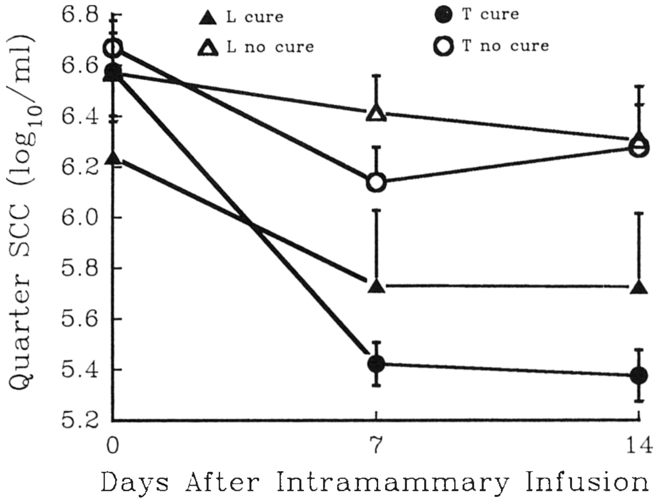
Figure 1 Effect of lactobacillus (L) or cephapirin (T) intramammary infusion of quarters with clinical mastitis on quarter SCC.



while mean SCC remained unchanged in the L quarters. Infected and uninfected quarters showed similar trends. There was not a decrease in SCC ($P > .05$) for either infected or uninfected quarters treated with lactobacillus. The SCC for both infected and uninfected quarters decreased ($P < .05$) from day 0 to 7 for T quarters. Similar results were obtained when infections were grouped according to major and minor pathogens. The SCC ($\bar{X} \pm$ S.E.) of L quarters was higher than T quarters at day 7 for both major (6.20 ± .17 and 5.85 ± .11 log_{10}/ml, respectively, $P = .09$) and minor (6.11 ± .35 and 5.30 ± .14 log_{10}/ml, respectively, $P = .06$) pathogen infections.

Quarter SCC were lower ($P < .05$) for cured than uncured clinical quarters for both treatments at day 7 although there was no difference ($P > .05$) at day 0 (Figure 2). Somatic cell counts for cured and uncured quarters did not differ 14 days after treatment with L. However, mean SCC for cured quarters was less than uncured quarters for T at day 14 after treatment. There was no change ($P > .05$)

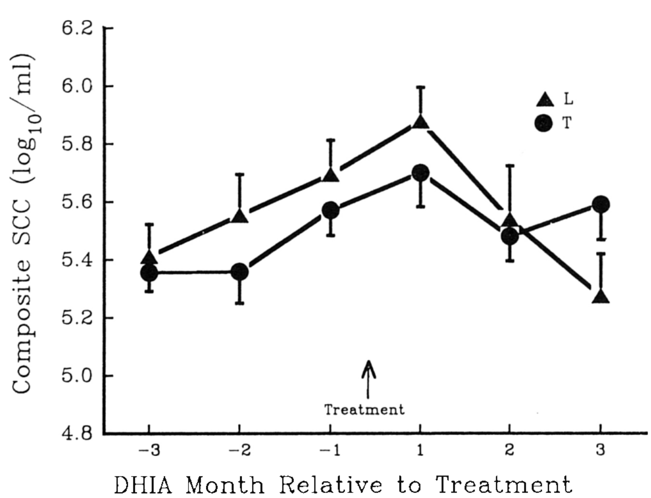
Figure 2 Effect of lactobacillus (L) or cephapirin (T) intramammary infusion of bacteriologically-positive clinical quarters on quarter SCC categorized according to cured or uncured quarters.



in SCC for uncured quarters from day 0 to 7 for either treatment. The reduction in SCC for cured quarters from day 0 to 7 was less ($P < .05$) for L and T quarters.

There was no difference between treatments ($P > .05$) for DHIA composite SCC prior to and following treatment (Figure 3). Composite SCC for both treatment groups were higher for the month following treatment and returned to pretreatment level the following month. Although the increases following treatment were not different between experimenal groups ($P > .05$), the increase within L group was significant ($P < .05$).

Figure 3 Effect of lactobacillus (L) or cephapirin (T) intramammary infusion of quarters with clinical mastitis on Dairy Herd Improvement Association (DHIA) composite SCC.



Discussion

The difference in the number of clinical quarters in the two treatment groups was due to chance since animals were assigned to treatment based upon identification number status. The predominantly isolated bacteria were major pathogens. The bacteriological profile of quarters was similar to that reported previously by Hogan *et al* [4].

Cure rates of quarters treated with lactobacillus were lower than those treated with cephapirin. The cure rate for lactobacillus-treated quarters is similar to that reported previously[4,15,16] for cephapirin treatment but it is uncertain in the present study how many quarters would have recovered spontaneously. Hogan *et al.*[4] reported a 9.1% spontaneous cure rate of clinical quarters while Seymour *et al.*[15] found a 63% spontaneous recovery rate of subclinically infected quarters, with the majority of infections being coagulase-negative staphylococci. If we assumed a 9% spontaneous cure rate of the clinical quarters in the present study, the cure rate for lactobacillus would be only half that of cephapirin treatment (26 vs. 49%). The main contributor to the difference in cure rates between treatments was the cure rate of major pathogen infections, especially those infected with Gram-negative bacilli. None of the Gram-negative bacilli quarters treated with lactobacillus recovered while the majority of cephapirin-treated quarters were cured. The Gram-negative bacilli infection cure rate following cephapirin treatment was much higher than the 13% cure rate previously reported by Hogan *et al.*[4] This difference may be due to earlier identification and treatment of infected quarters which would likely result in a higher eradication success rate.[5,17] In agreement with previous studies,[4,15,16] minor pathogen cure rate was appreciably higher than major pathogen cure rate.

Mean quarter SCC remained unchanged following lactobacillus treatment, and decreased with cephapirin treatment. The decrease in SCC following cephapirin treatment has been reported previously[16]. The difference in SCC effect corresponds with the difference in cure rates between treatments. Although cure of infected quarters was reflected in reduced SCC at day 7, as reported previously,[15,16,18] the resulting SCC reduction was less for lactobacillus than for cephapirin-treated quarters. As reported by Lindstrom *et al.,*[19] composite SCC was not a good indicator of individual quarters SCC response due to the masking effect of milk from other quarters.

These results do not support the speculation that this type of lactobacillus product is effective in the treatment of clinical mastitis. Cure rates were low and SCC remained unchanged following treatment with lactobacillus. Cure rates and SCC reduction in clinical quarters receiving an intramammary infusion of a cephapirin product were greater than those of clinical quarters treated with the lactobacillus product.

Summary

The effects of treating clinical mastitis with intramammry infusions of either a lactobacillus or an antibiotic preparation on intramammary infection cure rate and on milk somatic cell count (SCC) were compared during a 10 month study. A total of 75 clinical quarters were randomly assigned to one of two treatments. Quarter foremilk samples were obtained from all quarters at day 0, 7, and 14 following infusion to determine the microbiological status and SCC. Monthly composite milk SCC were obtained from the Dairy Herd Improvement Association (DHIA). The majority of pathogens isolated were gram-negative bacilli and enviornmental streptococci. Treatment of quarters with lactobacillus cured 35.3% of infected quarters, while 57.7% of infections treated with antibiotic were eliminated. Correspondingly, average SCC remained unchanged for lactobacillus-treated quarters for 14 days after infusion while average SCC of antibiotic treated quarters decreased during the same time period. Although there was no treatment difference in monthly DHIA composite SCC prior to and following treatment, there was an increase in composite SCC for lactobacillus-treated quarters. The results indicate that this lactobacillus product was not effective as an intramammary treatment for mastitis.

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