

Evaluation of frequent milkout for treatment of cows with experimentally induced *Escherichia coli* mastitis

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Objective—To evaluate the effect of frequent milkout (FMO) on the outcome of experimentally induced *Escherichia coli* mastitis in cows.

Design—Randomized complete block study.

Animals—16 Holstein dairy cows.

Procedure—Cows were randomly assigned to 1 of 4 groups and were either not infected and not treated (NI-NT), experimentally infected with *E coli* and not treated (EC-NT), not infected and FMO (NI-FMO), or experimentally infected with *E coli* and FMO (EC-FMO). The infected quarter in cows in FMO groups was milked out every 4 hours from 16 to 36 hours and every 6 hours from 36 to 84 hours after challenge, with the aid of oxytocin administration. Somatic cell counts (SCC); times to bacterial, clinical, and systemic cures; and serum concentrations of α -lactalbumin were determined.

Results—Use of FMO did not appear to affect SCC. For EC-NT and EC-FMO groups, mean bacterial cure times were 203 and 159 hours, clinical cure times were 276 and 360 hours, and systemic cure times were 144 and 159 hours, respectively; these times were not significantly different. Concentrations of α -lactalbumin were significantly increased in the EC-NT group at 12 hours and in the NI-FMO group at 36 and 60 hours after challenge, compared with values of cows in other treatment groups.

Conclusions and Clinical Relevance—Compared with results in control cows, FMO does not appear to be an efficacious treatment for experimentally induced moderate to severe *E coli* mastitis. (*J Am Vet Med Assoc* 2003;222:63–66)

Mastitis is an economically important disease, and costs of clinical mastitis have been estimated at more than \$100/case.¹ On dairy farms with bulk-tank milk somatic cell counts (SCC) < 150,000 cells/mL, 35 to 55% of cows had at least 1 episode of clinical mastitis,^{2,4} and the greatest proportion of milk samples

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with positive results of bacteriologic culture from those cows yielded coliform bacteria.^{2,5}

There is no consensus for the most appropriate treatment for coliform mastitis. Frequent milkout (FMO) was encouraged as treatment for mastitis as early as 1869 according to Sloan⁶: “The bad milk should be drawn three to four times a day, for by remaining in the bag, it tends to increase the inflammation.” Since that time, this practice, often facilitated by injection of oxytocin, has gone in and out of favor. Use of antimicrobials for treatment of coliform mastitis has been discouraged in recent years because of the lack of proven efficacy, risk of antimicrobial residues in bulk-tank milk and resulting penalties, and more recently, recognition of development of antimicrobial resistance of bacterial pathogens.

Current teaching encourages supportive therapy (fluids, calcium, anti-inflammatory drugs) and the potential, but not proven, removal of endotoxins through FMO.⁷ In a recently completed study, the outcome of naturally occurring clinical coliform mastitis was not improved in 10 cows that were frequently milked out following administration of oxytocin, compared with 9 cows that were not frequently milked out.⁸ The purpose of the study reported here was to evaluate the effect of FMO on the outcome of experimentally induced *Escherichia coli* mastitis in cows. Our hypothesis was that frequent milking out of quarters experimentally infected with *E coli* would not enhance return to normal health status and would not affect or consistently improve times to either bacteriologic, clinical, or systemic cure.

Materials and Methods

Cows—Sixteen Holstein cows from the Virginia Tech Dairy Center were equally and randomly assigned to 1 of 2 blocks of 8 cows in a randomized complete block experimental design. Cows were selected from 212 cows on the basis of days in milk and being free of clinical mastitis during the current lactation. Selected cows were free of bacterial infection in 4 quarter samples collected prior to assignment to the study. Average days in milk were 172 days (range, 56 to 243 days), average lactation number was 2 (range, 1 to 5), and average daily milk production was 69.5 lb (range, 46 to 88 lb). Cows in each block were randomly assigned by choosing numbers out of a hat to 1 of 4 groups: not experimentally infected and not frequently milked out (not infected, not treated [NI-NT]), experimentally infected with *E coli* and not treated (EC-NT), not experimentally infected but frequently milked out (NI-FMO), and experimentally infected with *E coli* and frequently milked out (EC-FMO). The experiment was initiated at the beginning of July 1999 for cows in block 1 and the beginning of August 1999 for cows in block 2. Cows were housed separately from the main

herd in freestalls bedded with sawdust, milked twice per day at 6 AM and 6 PM in stanchions with a bucket milker, and fed a total mixed ration in a drive-through bunk. Cows were given a 3-day acclimation period to the new environment and milking system prior to experimental challenge. An *E coli* vaccination program had not been used in the herd.

Experimental infection—The *E coli* 727 suspension for intramammary challenge was prepared by use of a published procedure⁹ modified as follows. An isolated colony of *E coli* 727⁹ was placed on 5% sheep blood agar and incubated at 37°C for 24 hours. One isolated colony was transferred into brain-heart infusion broth and incubated at 37°C for 12 hours. The broth culture was centrifuged at 3,000 × g for 5 minutes, and the pellet was resuspended in sterile phosphate-buffered saline (PBS) solution. Serial dilutions of the bacterial suspension were made in PBS solution, and 50 µL of each dilution was plated on McConkey agar and incubated for 24 hours at 37°C. Bacterial suspensions with colony counts ranging from 50 to 100 colony-forming units (CFUs)/mL were selected. Five aliquots of 1 mL each were prepared, of which 4 aliquots were used for infusion into the right front quarter of challenge-group cows, and 250 µL of the remaining aliquot was immediately plated on McConkey agar for determination of bacterial challenge dose. Challenge dose was 84 CFUs/mL for cows in block 1 and 48 CFUs/mL for cows in block 2.

Frequent milkout schedule—The right front quarters of the NI-FMO and EC-FMO cows were milked out by hand at 4-hour intervals from 12 to 36 hours after challenge and at 6-hour intervals from 36 to 84 hours after challenge. Oxytocin (2 mL, 20 U/mL) was administered IV or IM at each milkout to facilitate milk removal.

Sampling protocol—Cows were monitored and sampled (Table 1). Physical examinations included measurement of rectal temperature, pulse rate, respiration rate, hydration status, evaluation of rumen motility and strength, and appearance of milk or udder secretion; cows were considered systemically ill if 2 or more of these clinical parameters were abnormal.⁸ Prior to experimental challenge, milk samples for bacteriologic culture and somatic cell counts were collected at the morning milking. Production data were recorded -3, -2, -1, and 0 days prior to challenge and at each treatment and milking after challenge.

Microbiologic procedures—All milk samples were collected aseptically after wiping the teat ends with a gauze pad soaked in isopropyl alcohol. Samples were collected in sterile milk sample vials. Teats were dipped with a 1% iodine-based barrier teat dip after sample collection and milking. Milk samples (50 µL) were plated on 5% sheep blood agar and incubated for 24 hours at 37°C. The number of CFUs per milliliter were determined. Identification of isolates from

initial cultures was confirmed by use of biochemical test strips.^a

Somatic cell counts—Somatic cell counts (SCC) were determined by use of an automated analyzer^b at the Dairy Herd Improvement Association laboratory at the Virginia Polytechnic Institute. California mastitis test^{10,c} results were used when electronic counts were missing (mostly because of insufficient volume), and the resulting variable for analysis was designated adjusted SCC (SCCA).

α-Lactalbumin analysis—Blood for α-lactalbumin analysis was drawn from the coccygeal vein with an 18-gauge, 1-in needle into a plain, 10-mL, evacuated tube. The blood was centrifuged, and serum was removed and frozen for later analysis. α-Lactalbumin concentrations were determined by use of radioimmunoassay, as described.¹¹

Time to cure—Time to bacterial cure was defined as the time interval in hours from experimental challenge to obtaining the first milk sample that yielded negative culture results followed consistently with negative culture results for all subsequent samples. Time to clinical cure and time to systemic cure were defined as the time intervals from experimental challenge to return to consistent normal clinical findings in the challenged quarter and return to reference ranges of all physical examination values, respectively.⁸

Statistical analyses—Effects of *E coli* infection and FMO on hours to bacterial cure, clinical cure, and systemic cure were tested by use of an ANOVA using the general linear models procedure of a statistical software program.^d Log-transformed SCCA and α-lactalbumin data were analyzed by use of repeated measures ANOVA. Only values at times when all groups were represented were used in the analysis. Least squares means are reported. Covariation among repeated measurements on the same cow were modeled by use of a first-order autoregressive model. Significant interactions were evaluated for simple main effects with a Bonferroni correction to maintain α ≤ 0.05. Analysis of study power (the calculation of the probability of finding a significant difference at α = 0.05) for bacterial cure, clinical cure, and systemic cure was performed with a statistical package.^{12,e}

Results

Cows experimentally challenged with *E coli* became systemically ill 14.5 hours (range, 12 to 20 hours) after experimental challenge. Mean ± SD peak temperature was 40.9 ± 0.8°C (105.6 ± 1.5°F), mean ± SD peak pulse rate was 92 ± 27.8 beats/min, and mean ± SD peak respiration rate was 66.5 ± 15.3 breaths/min during the period when cows were systemically ill. Cows in the NI-NT and NI-FMO groups did not

Table 1—Sampling protocol for cows assigned to 1 of 4 groups (n = 4/group) to evaluate effectiveness of frequent milkout (FMO) for treatment of mastitis. Cows were either not infected (NI) or experimentally infected with *Escherichia coli* (EC) at hour 0, and either not treated (NT) or treated with FMO from hours 12 to 84

Group	Time (h)																									
	-168	-72	-48	-24	0	4	8	12	16	20	24	28	32	36	42	48	54	60	66	72	78	84	96	108	156–828*	
NI-NT	A	A	A		B	C	D	D			C			C		C		C		C		C		C		A
EC-NT,																										
NI-FMO & EC-FMO	A	A	A	B	C	D	D	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A

*Samples obtained every 168 hours (7 days) from 156 through 828 hours.
A = Times when samples were obtained for the California mastitis test, bacteriologic culture, and somatic cell counts. B = Times when blood samples were collected for determination of serum α-lactalbumin concentrations in addition to tests denoted in A. C = Times when samples were obtained for tests denoted in A and B and physical examinations were performed. D = Times when only physical examinations were performed.

become systemically ill or have abnormal milk, except for 1 cow in the NI-NT group in block 2 with increased SCCs starting at hour 84; no microbial organisms were cultured from those samples.

Cows experimentally challenged with *E. coli* in block 1 all had severe systemic illness⁸ by 12 hours after challenge, and abnormal milk was detected between 12 and 20 hours after challenge. Cows experimentally challenged with *E. coli* in block 2 became moderately to severely ill within 20 hours after challenge, and abnormal milk was detected between 16 and 20 hours after challenge. The range of temperature, pulse, and respiration for *E. coli*-challenged cows was 37.8 to 41.6°C (100.0 to 106.8°F), 60 to 112 beats/min, and 40 to 110 breaths/min, respectively. Cows in block 2 had been challenged with a reduced dose (48 CFUs/mL) to achieve moderate systemic involvement in order to avoid the severe systemic effects seen in cows infected with 84 CFUs/mL in block 1. Neither cows in block 1 nor block 2 were treated with systemic or intramammary administration of drugs. Milk yields were reduced in cows infected with *E. coli* ($P = 0.004$) but did not appear to be affected by FMO. All experimentally challenged cows recovered fully without additional treatment.

Mean times to bacterial cure, clinical cure, and systemic cure were not different between groups EC-NT and EC-FMO. For EC-NT and EC-FMO groups, mean times to bacterial cure were 203 and 159 hours, respectively ($P = 0.53$), mean times to clinical cure were 276 and 360 hours, respectively ($P = 0.64$), and mean times to systemic cure were 144 and 159 hours, respectively ($P = 0.95$).

The SCCAs in cows infected with *E. coli* were higher than in cows not infected with *E. coli* from 24 through 156 hours after experimental challenge (2.49×10^6 vs 0.096×10^6 cells/mL; $P < 0.001$; Fig 1), but were not affected by FMO ($P = 0.34$). Differences in

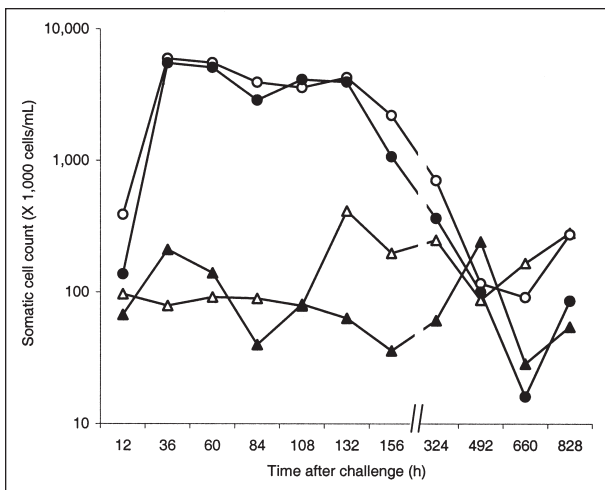


Figure 1—Adjusted somatic cell counts (antilog of least squares means of logarithmically transformed counts) at various times after experimental mammary infection with *Escherichia coli* in cows. Cows were either not infected and not treated (open triangles), experimentally infected but not treated (open circles), not infected and frequently milked out (solid triangles), or experimentally infected and frequently milked out (solid circles). From 24 to 156 hours after experimental challenge, the adjusted somatic cell counts of cows infected with *E. coli* were significantly ($P < 0.001$) higher than those of cows not infected.

SCCAs between groups at ≤ 12 hours after experimental challenge were not significant, irrespective of FMO, whereas SCCAs in the later weekly samples differed during the 4-week period depending on FMO group, irrespective of *E. coli* infection group ($P = 0.02$).

Serum α -lactalbumin concentrations were higher in EC-NT cows than in NI-NT, NI-FMO, and EC-FMO cows at 12 hours after experimental challenge, and higher in NI-FMO cows, compared with cows in the other groups at 36 ($P < 0.001$) and 60 ($P = 0.02$) hours after experimental challenge. Serum α -lactalbumin concentrations for NI-NT and EC-FMO were consistently $< 2 \mu\text{g/mL}$, whereas both the EC-NT and NI-FMO groups had cows with values $> 2 \mu\text{g/mL}$. These increased α -lactalbumin values were obtained at 12 and 24 hours after challenge for the EC-NT group and at 24 to 48 hours for the NI-FMO group.

Discussion

Frequent milkout is assumed to improve the clinical outcome of mastitis by removing bacteria, their toxins, or both. Results of the limited experimental study reported here do not support that assumption. Frequent milkout with administration of oxytocin did not decrease hours to clinical cure or systemic cure but may decrease hours to bacterial cure. However, producers usually do not know the results of culture prior to treatment, and their treatment efforts are directed toward clinical cure rather than bacterial cure. In this study, FMO did not decrease time to clinical cure. Frequent milkout did not appear to affect SCCs in the groups that were experimentally challenged with *E. coli*. Because the power of the study was low, we did not expect significant differences unless FMO was vastly superior to no treatment.

No benefits of FMO following oxytocin administration were reported when FMO and no FMO were compared in 19 cows with naturally occurring *E. coli* mastitis,⁸ or when the use of oxytocin and FMO were used in cows with acute mastitis.¹³ These reports, which appear to be the only studies in which the effect of FMO was the only factor of interest, support our results.

Frequent milkout may be as beneficial as intramammary administration of certain antimicrobials.¹⁴ In 1 report,¹⁴ 4 cows with clinical *E. coli* mastitis that received FMO following oxytocin administration and 2 of 3 cows treated with antimicrobials were free of *E. coli* infection by 3 to 4 weeks after infection. Treatment had no substantial effect when cows with naturally occurring *E. coli* mastitis were treated systemically with either gentamicin or erythromycin, compared with FMO and oxytocin administration several times per day and intramammary administration of cephalosporin.¹⁵ Differences in clinical cure or bacterial cure were not reported in cows with mild clinical mastitis that were treated with either intramammary administration of antimicrobials or oxytocin at milking to improve milkout at regular milking times.¹⁶

Cows with naturally occurring *E. coli* mastitis, which were treated with varying milkout frequencies following oxytocin administration and either intramammary administration of cephalosporin or intramammary

and systemic administration of oxytetracycline (on the basis of severity of mastitis), had no differences in bacterial or clinical cure between antimicrobial treatment groups.¹⁷ There was no significant difference in the number of cows treated with antimicrobials that were still clinically infected by the tenth milking after onset of clinical mastitis, compared with those that were not treated with antimicrobials. When clinical cure was evaluated in cows from which streptococci or coliform bacteria were isolated, antimicrobial-treated cows had a higher clinical cure rate by the tenth milking than did nontreated cows. Most quarters with coliform bacteria were free of bacteria by 14 days, irrespective of treatment group. Results of these studies lend support to our results in that irrespective of treatment, no significant differences in the outcome of cows with experimentally or naturally occurring *E coli* mastitis were recorded.

Most studies, as well as our study, were done on low numbers of cows of varying parities and stages of lactation. The power of this study to detect significant differences in time to cure was low. However, our goal was to determine whether FMO resulted in clinically relevant improvement in outcome; the results we obtained do not encourage us to either carry out or recommend more extensive studies.

α -Lactalbumin is a protein found in milk that can be used as an indicator of udder development and udder health.¹⁸ It enters the bloodstream either through compromised tight junctions, which are located between the mammary epithelial cells that line the alveolar lumen, through damaged alveolar epithelial cells, or through gaps left by sloughed epithelial cells.¹⁸ Stages of gestation and lactation, frequency of milking, and udder health status affect leakage of α -lactalbumin into blood.¹⁹ Serum α -lactalbumin concentration is positively correlated ($r = 0.60$) with milk SCC, and cows challenged with *E coli* endotoxins have increased SCCs, as well as increased serum α -lactalbumin concentrations.¹⁸ We expected increased serum α -lactalbumin concentrations in cows that were experimentally challenged with *E coli* and had increased SCCs. However, the highest α -lactalbumin concentrations were measured in the NI-FMO group at 36 and 60 hours after experimental challenge. Lower α -lactalbumin concentrations were expected with FMO, because cows that were milked 3 times per day were reported to have lower serum α -lactalbumin concentrations than cows milked twice per day.²⁰ Increased intramammary pressure attributable to the frequent stimulation¹⁹ but with selective milkout of 1 quarter only may have led to increased serum α -lactalbumin concentrations in NI-FMO cows.

Although this study was performed on a small number of cows, we conclude that FMO as a treatment for *E coli* mastitis does not improve outcome nor does it appear to have a negative effect, which is consistent with an earlier study⁸ of naturally occurring *E coli* mastitis.

^aAnalytical Profile Identification 20E, bioMerieux Vitek Inc, Hazelwood, Mo.

^bFossomatic 360, Foss North America, Eden Prairie, Minn.

^cCalifornia Mastitis Test, Jorgensen Laboratories Inc, Loveland, Colo.

^dSAS, v7.1, SAS Institute Inc, Cary, NC.

^ePASS, v6.0, NCSS, Kaysville, Utah.

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