Mild to Moderate Clinical Mastitis: Efficacy of Intramammary Amoxicillin, Frequent Milk-Out, a Combined Intramammary Amoxicillin, and Frequent Milk-Out Treatment Versus No Treatment

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ABSTRACT

Study objectives were to determine the efficacy of 4 methods (intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin and frequent milk-out, and no treatment) of managing mild to moderate clinical mastitis in a university dairy herd. Clinical and microbiological cures, milk production, disease progression, and California Mastitis Tests scores were evaluated. Cows meeting the study criteria were assigned to one of four treatment options based on a systematic randomization scheme (blocked by stage of lactation). Treatments were initiated prior to knowledge of culture results. Cows were observed and evaluated on d 1 to 8, 15, 22, 29, and 36. Overall, treatments were not significantly different than controls. However, when efficacy was evaluated by pathogen group, differences were observed. Intramammary amoxicillin appeared to be the most efficacious treatment for environmental streptococci. Frequent milk-out appeared to be detrimental for environmental streptococci. Treatment method appeared to have little effect on *Escherichia coli* mastitis, as nearly all cases recovered within a short time frame. None of the treatments were satisfactory for cases of *Klebsiella* mastitis. When obtained in a timely manner, treatment selection for clinical mastitis should be based on culture results.

(Key words: clinical mastitis, frequent milk-out, treatment, mastitis cure)

Abbreviation key: CI = confidence interval, CMT = California Mastitis Test, FMO = frequent milk-out, IMMA = intramammary amoxicillin.

INTRODUCTION

Mastitis treatment is the most common cause of antibiotic use in adult dairy cows and the most common cause of illegal antibiotic residues (Guterbock, 1993). Although much research has been conducted in clinical mastitis treatment, a consensus regarding treatment has never been achieved. One reason for the lack of consensus is that truly controlled studies with non-treated controls have not been conducted. Thus, most treatment regimes are anecdotal. Many studies have utilized “positive” controls. The use of positive controls (most often an approved intramammary antibiotic) will usually result in one treatment being better than the other. Although often assumed, there is usually no published evidence that either treatment is better than no treatment. Previous studies suggest that antibiotics may be ineffective in treatment of mastitis (Erskine et al., 1991; Guterbock et al., 1993). Some dairy producers and veterinarians have elected to not use antibiotics in their mastitis treatment protocol (Guterbock, 1993; Ashley, 1994). Ashley (1994) reports no loss of quarters or cows over the 4 yr in which no antibiotics were used to treat all clinical mastitis cases. Yet, this protocol may lead to severe clinical mastitis outbreaks with high bulk tank SCC (Cattell, 1996). Because there are so many agents of mastitis, it is unrealistic to expect a single treatment protocol to be effective for all mastitis cases. Most studies evaluating the efficacy of antibiotics in treatment of clinical mastitis have not had non-treated control cows. Thus, it remains unclear as to whether antibiotic therapy for clinical mastitis is better than no treatment.

Several non-antibiotic methods have been suggested for the treatment of clinical mastitis, such as frequent milk-out (FMO) with and without oxytocin, non-steroid anti-inflammatory drugs, steroids, fluids, hydrotherapy, intramammary infusions of saline, antihistamines, diuretics, hot and cold packing, hypertonic saline infusion, ultrasonic therapy, and other supportive therapy. A popular recommendation for clinical masti-

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Table 1. Criteria used to classify the severity of clinical mastitis.¹

Parameter evaluated	Normal	Abnormal
Rectal temperature (ambient temperature >75°F)	≤104°F	>104°F
Rectal temperature (ambient temperature ≤75°F)	≤103°F	>103°F
Heart rate/min ²	≤90	>90
Respiratory rate/min (ambient temperature >75°F) ²	≤90	>90
Respiratory rate/min (ambient temperature ≤75°F) ²	≤40	>40
Hydration status ³	<5%	≥5%
Rumen contraction strength ⁴	Strong	Weak or atonic
Secretion color/consistency	All others	Clear yellow ⁵

¹Severe mastitis = 2 or more abnormal parameters; moderate mastitis = one abnormal parameter; mild = no abnormal parameters.

²Assessments were made taking into account that both excitement and exercise may elevate both the respiratory and heart rates. High humidity may elevate the respiratory rate.

³Hydration status was determined by skin tenting of the neck and eyelid.

⁴Rumen strength was assessed by placing the stethoscope into the left paralumbar fossa while leaning into the cow. A strong rumen contraction will push the listener out 1 to 2 in.

⁵Clear, yellow-colored lacteal secretion with no apparent milk.

tis is milking the affected quarters out several times a day (Eberhart et al., 1987). In theory, FMO of the affected quarters helps remove the infectious agent and toxic products of the infection. Although there are several studies comparing treatment efficacy between two or more antibiotics, to our knowledge there are only a few studies comparing FMO to any other form of treatment. If FMO is beneficial, then the next step would be to determine the minimum number of FMO that are beneficial, because this method of treatment can be labor intensive and can increase labor costs. A combination of intramammary antibiotic therapy and FMO may be more beneficial than either method alone.

This study is unique, as there is a non-treated control group with which all other groups were compared. This is the first study that compares FMO with antibiotic therapy and untreated controls. The purpose of our study was to determine the efficacy of three methods (intramammary amoxicillin [IMMA], FMO, and FMO + IMMA) for treatment of mild to moderate clinical mastitis and compare them with untreated controls.

MATERIALS AND METHODS

Study Animals

The study was conducted over a 2-yr period utilizing lactating Holstein and Jersey cows from a university dairy with prior protocol approval of the University Animal Care Committee. Cows were milked twice a day and housed in freestalls bedded with shavings or straw. Some of the cows were maintained on grass pastures during the summer months. The rolling herd SCC was consistently <200,000 cells/mL. Pre-milking udder preparation consisted of application of an 0.25% iodine-based teat disinfection, forestripping, and teat drying with single service paper towels. A 1% iodine-based teat

disinfection was applied post-milking. Milking units were flushed with an automatic backflush system between cows. All cows were treated intramammarily at drying off with cephalixin. No bST or coliform vaccines were being used at the initiation of the study or during the study. Cows with naturally occurring clinical mastitis were detected by dairy personnel during routine milking procedures. Eighty-six percent of cases were in Holsteins, and 14% were in Jersey cows. A study collaborator examined the cow with clinical mastitis to classify the cow's condition as mild to moderate or severe based on the protocol in Table 1. Cows with clinical mastitis and one or fewer abnormal parameters were classified as having mild to moderate clinical mastitis and were used in the current study. Cows without evidence of clinical mastitis at the time of initial examination were not included in the study. Systemically ill cows with more than one abnormal parameter at the time of initial examination and cows with other concurrent diseases that required treatment were excluded. Cows with clinical mastitis that were included in the study were eligible for a new case of clinical mastitis 36 d after the previous case, provided that a microbiological cure occurred. If a cow was previously used in the study and a microbiological cure was not obtained, the cow was not eligible for inclusion again unless a different quarter was involved.

Treatments and Method of Treatment Assignment

Cows that met the criteria were systematically assigned to 1 of 4 treatments after being stratified by stage of lactation. Stage of lactation was categorized as 0 to 90 DIM, 91 to 180 DIM, and >180 DIM. Prior to initiation of the study, the 4 treatments were randomly identified as Treatments 1 to 4. Treatment 1 consisted

of IMMA (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA) administered according to manufacturer's directions (intramammary infusion every 12 h for a total of 3 treatments). Treatment 2 consisted of no treatment of any kind. Treatment 3 consisted of FMO of the affected quarters at least 2 h apart during the day for 3 d in addition to the 2 regular milkings in the parlor for a total of 6 milk-outs per infected quarter per 24-h period. Twenty IU of oxytocin were used intramuscularly to aid each milk-out. Treatment 4 consisted of a combination of Treatments 1 and 3 (FMO + IMMA). Intramammary amoxicillin was given only after the night milking (0100 to 0400 h) for 3 treatment periods (24-h intervals). Frequent milk-out began at least 8 h after IMMA therapy following the protocol of Treatment 3. The first cow on the study within a DIM stratum received Treatment 1. The second cow on the study received Treatment 2. The third cow on the study received Treatment 3. The fourth cow on the study received Treatment 4. The fifth cow on the study, within the same stratum, received Treatment 1 and so forth. All treatments were assigned and initiated prior to knowledge of culture results. If a cow had more than one clinical quarter, all quarters received the same treatment.

Assessment Days and Parameters

Study cows were assessed prior to milking on d 1 to 8, 15, 22, 29, and 36. In addition to the parameters from Table 1 (used to assess progression to systemic illness), the California Mastitis Test (CMT) was scored for each quarter. Milk from the affected quarters was assessed for a clinical cure by stripping milk through a strip cup screen, and a milk sample was aseptically collected from the affected quarters to assess microbiological cure. All teats were dipped with an iodine-based germicide (Blockade; West Agro, Inc., Kansas City, MO) after each assessment and each milk-out.

Cure Definitions and Microbiological Methods

Outcome variables examined were clinical cure, microbiological cure, progression to systemic illness, CMT scores at d 36, and daily milk production. A clinical cure was defined as no clots or flakes in the milk for 3 consecutive assessment d or 2 consecutive wk without a relapse. A microbiological cure was defined as no growth of the originally isolated pathogen for 3 consecutive assessment d or 2 consecutive wk without a relapse. Clinical and microbiological cures were evaluated on d 7 and 36. Day 7 represented the first day after the end of the withdrawal period for amoxicillin (60 h) for the FMO + IMMA group. Day 36 was chosen as the end

Table 2. Clinical mastitis culture results by organism.

Organism	n	%
<i>Streptococcus uberis</i>	30	29.1
<i>Escherichia coli</i>	20	19.4
<i>Klebsiella pneumoniae</i>	15	14.6
No growth	12	11.7
CNS	5	4.9
<i>Klebsiella oxytoca</i>	4	3.9
<i>Serratia marcescens</i>	4	3.9
<i>Streptococcus dysgalactiae</i>	3	2.9
<i>Staphylococcus aureus</i>	3	2.9
Yeast	3	2.9
<i>Citrobacter</i> spp.	1	1.0
<i>Enterobacter</i> spp.	1	1.0
<i>Enterococcus</i> spp.	1	1.0
<i>Streptococcus mutans</i>	1	1.0
Total	103	100

point of surveillance for a case. If microbiologic results were negative at the initial sampling and thereafter, then time to microbiological cure was considered 1 d. Fifty microliters of milk were plated on blood agar immediately upon return to the mastitis laboratory, usually within 30 min. Cultures were incubated for 24 to 48 h at 37°C. All organisms were initially identified by colony characteristics. Gram-negative organisms were identified by biochemical test strips (API 20E; bioMérieux Vitek, Inc., Hazelwood, MO). Streptococcal organisms were identified by catalase, Gram-stain, esculin, and CAMP reactions. Staphylococcal organisms were differentiated by the tube-coagulase test and hemolytic patterns. Unusual organisms were identified by Gram-stain (yeast) or were submitted to the Virginia-Maryland Regional College of Veterinary Medicine Clinical Bacteriology Laboratory. Milk weights were collected at each milking and were used to assess the percentage of decreased production caused by mastitis.

Statistical Analysis

Descriptive statistics were performed on data by treatment and class of organisms recovered. Data were analyzed using SAS (1990) and EGRET (1993). The effect of treatment group or culture result on dichotomous outcome variables, such as clinical or microbiological cure or new infection, were analyzed at the cow level using logistic regression. For cows with multiple infected quarters, the cow was classified as cured if all affected quarters met the definitions for a clinical or microbiological cure. The effect of treatment on milk production was analyzed using repeated measures ANOVA. Continuous outcome variables (time to clinical cure and time to microbiological cure) were tested for significance using mixed linear models with blocking factors for stage of lactation included as random effects. Quarter data were analyzed using the Fisher Exact

test (2×2 tables) at the Simple Interactive Statistical Analysis website (<http://home.clara.net/sisa/>).

RESULTS

Descriptive Statistics

Eighty-five clinical mastitis events of 70 cows (99 total affected quarters) were eligible for the study. An event was defined as clinical mastitis in one or more quarters at a single point in time. Fifty-seven cows had one mastitis event, 11 cows had 2 events, and 2 cows had 3 events. The minimal interval between consecutive events of clinical mastitis in the same cow was 59 d, and the median interval was 8.5 mo. Two-thirds of the repeated events occurred in different lactations. One quarter was affected for 73 of the mastitis cases studied, 2 quarters for 11 cases, and 4 quarters for one case.

The distribution of lactations for cases included in the study was 34% first lactation, 26% second lactation, and 40% in third lactation or greater. Twelve percent of the study events occurred from December to February, 21% from March to May, 38% from June to August, and 29% from September to November. The DIM at the time of diagnosis ranged from 1 to 452 (median = 120). None of these factors were significantly different among treatment groups ($P > 0.5$).

Seven percent (6 of 85) of mastitis events resulted in a cow becoming systemically ill after entering the study (two *Klebsiella pneumoniae*, one *Escherichia coli*, three *Streptococcus uberis*). Initial treatment of cows progressing to systemic illness was either FMO or IMMA.

Culture Results

Eighty-five events of clinical mastitis were eligible for treatment comparisons. There were 103 culture results because multiple quarters were infected in some cows, and two pathogens were isolated from 4 of the 99 infected quarters (Table 2).

Treatment Effect on Clinical or Microbiological Cure

There was no significant effect of treatment on the proportion of mastitis events with a clinical or microbial cure for all organisms combined or stratified by organism category ($P > 0.2$). Clinical and microbiological cures by cow mastitis event are presented in Table 3. Quarter-level clinical and microbiological cures by mastitis case and major pathogen categories are presented in Tables 4 and 5.

The greatest effect on clinical or microbiological cures was organism category rather than treatment. Coliform IMI, especially *E. coli*, appeared to be more likely to cure than environmental *Streptococci* or mixed infec-

Table 3. Cow clinical and microbiological cure by treatment and organism category.

Culture category	Untreated		IMMA ¹		FMO		FMO + IMMA		Total	
	% CC ²	% MC ³	% CC	% MC	% CC	% MC	% CC	% MC	% CC	% MC
No growth	100% (2/2)	100% (2/2)	0% (0/1)	100% (1/1)	50% (1/2)	100% (2/2)	50% (1/2)	100% (2/2)	57% (4/7)	100% (7/7)
<i>Streptococci</i>	40% (2/5)	40% (2/5)	50% (1/2)	50% (1/2)	25% (2/8)	25% (2/8)	38% (3/8)	25% (2/8)	35% (8/23)	30% (7/23)
Coliform ⁴	67% (6/9)	78% (7/9)	53% (8/15)	60% (9/15)	29% (2/7)	71% (5/7)	71% (5/7)	71% (5/7)	55% (21/38)	68% (26/38)
Mixed ⁵	100% (1/1)	0% (0/1)	100% (2/2)	100% (2/2)	0% (0/2)	0% (0/2)	0% (0/1)	0% (0/1)	50% (3/6)	33% (2/6)
Other ⁶	60% (3/5)	20% (1/5)	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/1)	100% (1/1)	100% (1/1)	63% (5/8)	38% (3/8)
Total	64% (14/22)	55% (12/22)	57% (12/21)	67% (14/21)	25% (5/20)	45% (9/20)	53% (10/19)	53% (10/19)	50% (41/82)	55% (45/82)

¹IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

²CC = Clinical cure by 36 d without relapse.

³MC = Microbiological cure by 36 d without relapse.

⁴Coliforms = 20 *Escherichia coli*, 17 *Klebsiella*, 1 *Citrobacter*, and 1 *Enterobacter*.

⁵Included 5 environmental *Streptococci* and coliform events and 1 environmental *Streptococci* and *S. aureus* event.

⁶Included 2 *Staphylococcus aureus*, 3 coagulase-negative staphylococci, and 3 yeast events.

Table 4. Percentage quarter clinical cure within 7 and 36 d by treatment and organism category.

	Untreated		IMMA ¹		FMO		FMO + IMMA		Total	
	7 d	36 d	7 d	36 d	7 d	36 d	7 d	36 d	7 d	36 d
No growth	100 (3/3)	100 (3/3)	67 (2/3)	67 (2/3)	33 (1/3)	67 (2/3)	33 (1/3)	33 (1/3)	58 (7/12)	67 (8/12)
<i>Streptococci</i>	29 (2/7)	43 (3/7)	40 (2/5)	75 (3/4)	17 (2/12)	22 (2/9)	9 (1/11)	45 (5/11)	20 (7/35)	42 (13/31)
<i>Escherichia coli</i>	100 (4/4)	100 (4/4)	67 (6/9)	100 (9/9)	67 (2/3)	67 (2/3)	33 (1/3)	100 (4/4)	68 (13/19)	95 (19/20)
<i>Klebsiella</i>	20 (1/5)	40 (2/5)	0 (0/7)	0 (0/6)	0 (0/2)	0 (0/1)	50 (2/4)	50 (2/4)	17 (3/18)	25 (4/16)

¹IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

tions (all included at least one environmental *Streptococci*). Logistic regression *P* values for the effect of organism category, controlling for treatment groups, were 0.01 for microbiological cure and 0.1 for clinical cure.

Microbial growth by quarter for the first 3 d after treatment initiation is presented in Table 6, which is divided into no growth, moderate growth (20 to 1700 cfu/mL), and high growth (>1700 cfu/mL). Eighty-seven percent of all pathogens had growth within the first 3 d after antibiotic therapy was initiated. When all pathogens were considered, FMO had a significantly higher percentage of high growth cases than untreated controls and IMMA. For coliforms, FMO had a significantly higher percentage of high growth cases than untreated controls and FMO + IMMA groups. Considering only *Streptococci*, the IMMA group had a significantly lower percentage of high growth cases than the FMO and FMO + IMMA treatment groups.

CMT

California mastitis test data are presented for the major pathogen categories (Table 8). When all results were combined, quarters treated with IMMA were significantly more likely to have a CMT score of "trace" (approximately 300,000 cells/mL) by d 36 than the FMO group (*P* < 0.05). The FMO group had the lowest percentage (21%) of quarters with a CMT score of "trace" by d 36 of all treatment groups. No significant differences were noted among streptococcal, coliform, or miscellaneous infected quarters (*P* > 0.10). Among quarters in which culture revealed no growth, 100% of quarters (3 of 3) in the non-treated group were "trace" by d 36

compared with 0% of quarters (0 of 3) in the FMO + IMMA group (*P* < 0.10).

Treatment Effect on New Infections in Previously Uninfected Quarters

Treatment tended to increase the risk of developing clinical mastitis in previously unaffected quarters within 4 d of starting treatment (logistic regression, *P* = 0.06 when controlling for organism category). The FMO + IMMA treatment showed the greatest effect with an odds ratios of 6.4 (95% confidence interval [CI]; 1.6, 30.6) for developing a new clinical quarter compared with non-treated cases. For IMMA and FMO treatments, the odds ratios for this comparison were 3.6 (95% CI; 0.9, 16.8) and 2.4 (95% CI; 0.6, 11.1), respectively.

Milk Production

There was a significant effect of treatment on milk production following mastitis (*P* < 0.001) (Figure 1). Adjusting for breed, lactation, DIM, month of study, and mastitis organism, cows in the untreated group had similar daily milk production before and after the mastitis event (Table 7). Decreases of 2.0 to 3.1 kg in production between the period before mastitis and 3 post-treatment periods were observed for FMO and IMMA treatment groups. A 1.9-kg drop was calculated for the FMO + IMMA group 30 d or more after the mastitis event.

Table 5. Percentage quarter microbiological cure within 7 and 36 d by treatment and organism category.

	Untreated		IMMA ¹		FMO		FMI + IMMA		Total	
	7 d	36 d	7 d	36 d	7 d	36 d	7 d	36 d	7 d	36 d
<i>Streptococci</i>	29 ^{ab} (2/7)	29 ^{ab} (2/7)	60 ^a (3/5)	75 ^{ab} (3/4)	8 ^b (1/12)	22 ^{ab} (2/9)	9 ^{ab} (1/11)	18 ^{ab} (2/11)	20 (7/35)	29 (9/31)
<i>Escherichia coli</i>	75 (3/4)	100 (4/4)	89 (8/9)	89 (8/9)	67 (2/3)	100 (3/3)	100 (4/4)	100 (4/4)	85 (17/20)	95 (19/20)
<i>Klebsiella</i>	40 (2/5)	60 (3/5)	14 (1/7)	29 (2/7)	67 (2/3)	67 (2/3)	50 (2/4)	50 (2/4)	37 (7/19)	47 (9/19)

¹IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

^{ab}Subcolumn means within row and day category with different superscripts differ (*P* < 0.10).

Figure 1. Average milk production relative to the date of mastitis by treatment group. (Note: Data were not adjusted for stage of lactation or season.)

DISCUSSION

Analyzing the data by genus and species (*E. coli* and *Klebsiella*) and major pathogen group (*Streptococci*) resulted in relatively small numbers per treatment group, thereby decreasing our ability to detect significant differences. However, the researchers felt that potentially relevant information would not be revealed if cases were analyzed as a whole rather than by the methodology used. Findings should not be considered conclusive but rather hypothesis generating for future studies utilizing greater numbers and multiple herds. Direct comparison of results with other clinical mastitis studies is difficult because standard definitions for clinical cure, microbial cure, systemic cure, and total cure have not been established nor has a standard severity scoring system for clinical mastitis been established. In a study by Morin et al. (1998), only 15 of 198 (7.5%) quarters were still clinical at 14 d and in a California study (Van Eenennaam et al., 1995); only 24 of 138 (17%) quarters were still clinical at 4.5 d. In the current study, 28 of 82 (34%) quarters were still clinical at 36 d. This fairly large difference may be related to time of evaluation of the cow or methodology used to determine clinical status. In the Morin et al. study (1998), the cows were

Table 6. Microbial growth during the first 3 d after treatment initiation.

Culture category	No growth after treatment	20 to 1700 cfu/mL	>700 cfu/mL
All pathogens ¹			
IMMA ²	16% (4/25)	44% (11/25)	40% ^a (10/25)
Untreated	17% (4/23)	48% (11/23)	35% ^a (8/23)
FMO	5% (1/22)	23% (5/22)	73% ^b (16/22)
FMO + IMMA	10% (2/21)	38% (8/21)	52% ^{ab} (11/21)
Total	12% (11/91)	38% (35/91)	49% (45/91)
<i>Streptococci</i>			
IMMA	40% (2/5)	40% (2/5)	20% ^a (1/5)
Untreated	29% (2/7)	14% (1/7)	57% ^{ab} (4/7)
FMO	8% (1/12)	8% (1/12)	83% ^b (10/12)
FMO + IMMA	9% (1/11)	9% (1/11)	82% ^b (9/11)
Total	17% (6/35)	14% (5/35)	69% (24/35)
Coliforms			
IMMA	13% (2/16)	50% ^{ab} (8/16)	38% ^{ab} (6/16)
Untreated	22% (2/9)	67% ^{ab} (6/9)	11% ^b (1/9)
FMO	0% (0/8)	25% ^a (2/8)	75% ^a (6/8)
FMO + IMMA	13% (1/8)	88% ^b (7/8)	0% ^b (0/8)
Total	13% (5/39)	59% (23/39)	31% (12/39)

^{a,b}Subcolumn means within column, growth, and pathogen heading with different superscripts differ ($P < 0.05$).

¹All pathogens excludes quarters yielding no growth.

²IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

Table 7. Production differences for 3 postmastitis event periods compared with pretreatment production, SE of the difference, and 95% confidence intervals (CI).

Treatment	Milk LSM ¹											
	-30 d			0 to 10 d			11 to 30 d			> 30 d		
				Difference	SE	95% CI	Difference	SE	95% CI	Difference	SE	95% CI
IMMA ²	25.4	22.8	22.3	-2.6	0.5	-3.5 to -1.7	-3.1	0.7	-4.5 to -1.8	-2.7	0.8	-4.3 to -1.2
FMO	25.9	23.3	23.4	-2.6	0.5	-3.6 to -1.6	-2.0	0.7	-3.4 to -0.6	-2.5	0.7	-4.0 to -1.1
FMO + IMMA	25.3	24.6	24.6	-0.6	0.5	-1.5 to 0.3	-0.7	0.7	-2.1 to 0.7	-1.9	0.7	-3.4 to -0.4
CX	25.9	26.0	25.4	0.1	0.5	-0.8 to 1.0	-0.5	0.6	-1.8 to 0.8	-0.7	0.7	-2.2 to 0.7

¹Adjusted for breed, lactation group, season of parturition, year-month of study, DIM category, and culture result category.²IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

evaluated within 30 min after milking; in the current study, cows were usually evaluated prior to milking. In the California study, assessment of clinical status was made by udder palpation and visual observation of fore-milk stripped on the floor. Further, most studies determine microbial cure based on only one or two follow-up cultures usually occurring within 4 wk post-mastitis episode. This methodology will result in some false-positive microbial cures. In addition, prior mastitis literature typically categorizes clinical mastitis as sub-acute, acute, or peracute (relating to a time frame) rather than by severity level as reported here. Although comparisons are made throughout this discussion, differences may be due to the lack of standards mentioned.

Clinical mastitis etiology was similar to findings of other studies with the exception that the percentage of no growth cultures tended to be lower (Smith et al., 1985; Bartlett et al., 1992; Morin et al., 1998). One reason for the lower percentage of no growth in the current study was probably due to daily culturing of affected quarters, which allowed greater confidence in infection status, even when only a few colony-forming units were present.

Treatment Effect on Clinical and Microbial Cure

We found no overall or organism-specific treatment effects on clinical or microbial cures when cow was used as the unit. The lack of significance for the analyses of individual pathogens was likely a result of small sample sizes for the analysis of treatment efficacy for specific pathogens. However, the results do suggest that treatment efficacy is different for different organisms when quarter was used as the unit.

Although FMO has been advocated as an accepted means of managing clinical mastitis, the data from this study did not support its use. Morin et al. (1998) demonstrated similar results; in that study, the cows' outcome when treated with antibiotics and FMO and supportive therapy was more favorable than that of cows treated with FMO and supportive therapy alone. The data from the current study suggest that FMO may be detrimental in cases of environmental streptococci. Both FMO and FMO + IMMA groups had fewer clinical and microbial cures than the untreated group. With the possible exception of *Klebsiella* species, FMO + IMMA did not appear to yield an improved outcome over the other 3 treatments. In a Czechoslovakian study, researchers recommended FMO based on study results of no significant difference between FMO-treated cows vs. those given antibiotics (Opletal et al., 1985). In that study, results were not evaluated by culture result, and cows that did not respond favorably to FMO after 48 h were included in the antibiotic-treated group. Frequent milk-

Table 8. California Mastitis Test scores less than "trace" at d 36 by culture result and treatment.

Culture result	CX	IMMA ¹	FMO	FMO + IMMA	All treatments
			(%)		
<i>Streptococci</i>	29 ^a (2/7)	75 ^a (3/4)	22 ^a (2/9)	27 ^a (3/11)	32 (10/31)
<i>Escherichia coli</i>	100 ^a (3/3)	89 ^a (8/9)	33 ^a (1/3)	100 ^a (3/3)	83 (15/18)
<i>Klebsiella</i>	20 ^a (1/5)	17 ^a (1/6)	0 ^a (0/1)	50 ^a (2/4)	25 (4/16)
No growth	100 ^a (3/3)	33 ^{ab} (1/3)	33 ^{ab} (1/3)	0 ^b (0/3)	42 (5/12)
Miscellaneous	29 ^a (2/7)	25 ^a (1/4)	0 ^a (0/3)	100 ^a (2/2)	31 (5/16)
All results ²	44 ^{ab} (11/25)	54 ^a (14/26)	21 ^b (4/19)	43 ^{ab} (10/23)	42 (39/93)

^{a,b}Subcolumn means within row and treatment category with different superscripts differ ($P < 0.10$).

¹IMMA = Intramammary amoxicillin (Amoxi-mast; SmithKline-Beecham Animal Health, Exton, PA); FMO = frequent milk-out.

²Seventeen miscellaneous culture results were included in the denominators.

out may also increase the time to clinical cure. This theory is partially supported by data in Table 4 in which most culture result categories took longer to achieve clinical cure if FMO or FMO + IMMA were the treatments.

Coliforms. We found that IMMA appeared to have little efficacy against coliform pathogens in that the untreated group had a higher microbial cure than the IMMA group. Such finding is similar to that of Guterbock et al. (1993) in which coliform bacterial cure for amoxicillin-treated quarters was 38.1% compared with 57.7% of oxytocin-treated quarters. Cows in the study of Guterbock et al. (1993) study were not frequently milked-out but were simply given oxytocin at the time of routine milking.

The majority of clinical mastitis studies demonstrate a high percentage of microbial cures for *E. coli* (Erskine et al., 1991). The aforementioned statement appears to be true for our study, as treatment appeared to have little benefit for mild to moderate *E. coli* mastitis as the majority of cases cured within 1 wk. In contrast, researchers in a Finland study, in which all cows received intramammary antibiotics, reported only a 71% bacterial cure for *E. coli* mastitis at 21 d (Pyörälä and Pyörälä, 1998). In the current study, the only cow with *E. coli* mastitis that did not cure bacteriologically by 36 d had received the IMMA treatment. Morin et al. (1998) stated that their results supported a role for antibiotic use even when most cows with coliform mastitis have only mild disease; however, their data are not presented by severity level nor are the data for the major coliforms (*E. coli* and *Klebsiella*) presented separately. In the current study, 85% (17 of 20) of cases of *E. coli* mastitis were cured bacteriologically by 7 d, regardless of treatment. Provided that the cow can be monitored for signs of systemic illness, our data support no treatment as the preferred means of managing mild to moderate *E. coli* mastitis.

None of treatments used in the current study provided satisfactory cure rates for *Klebsiella* mastitis.

Only a few studies were found that reported a cure rate for *Klebsiella*. A Wisconsin study reported no cures (0 of 3) at 14 d when treated with intramammary cephalosporin (Timms and Schultz, 1984), and a Finland study reported 50% bacterial cures at 3 wk post-treatment (Pyörälä and Pyörälä, 1998).

Environmental Streptococci. The use of amoxicillin according to label directions resulted in greater microbial cures for *Streptococci* than the other treatments (Table 5). The data from the current study suggest that IMMA may be important for the management of clinical mastitis caused by environmental *Streptococci* and that FMO or FMO + IMMA may be detrimental. Intramammary amoxicillin appeared superior to the other treatments, not necessarily because of the 75% microbial cure ($n = 4$) but more so because of the low cure rates for the other treatments (29, 22, and 18% for no treatment, FMO, and FMO + IMMA, respectively). Morin et al. (1998) reported a streptococcal cure rate of 71% for those receiving antibiotics and supportive therapy vs. only 28% for those cows receiving supportive therapy alone. Conversely, Guterbock et al. (1993) reported a lower environmental *Streptococci* bacterial cure of 46.2% (6 of 13) for cows receiving intramammary amoxicillin. Our streptococcal bacterial cure is also consistent with those reported in a recent review (36 to 90%) of therapy protocols for environmental streptococcal mastitis (Keefe and Leslie, 1997). Although bacterial cure rates of 60 to 80% would not be considered excellent, they are certainly better than reported cure rates for untreated cases of clinical environmental *Streptococci* mastitis (29% in the current study) ranging from 0 to 32% (Chamings, 1984; Hallberg et al., 1994; Hillerton and Kliem, 2002).

New Clinical Cases in Previously Uninfected Quarters

We found that treatment did have a significant effect on the development of clinical mastitis in previously

unaffected quarters within 4 d of starting treatment. The odds of a new clinical case were 2.4 and 6.4 times higher for cows in the FMO and FMO + IMMA groups compared with non-treated cows. Although oxytocin may not have been necessary for every cow in the FMO group, several cows would not have had a sufficient milk let down without the aid of oxytocin. We observed that when some cows were injected with oxytocin, non-clinical quarters would sometimes leak milk. This might predispose these quarters to new IMI and possibly clinical mastitis. All teats were dipped with a germicide after every FMO, but some non-clinical quarters continued to leak milk. The odds of a new clinical case occurring in cows in the IMMA treatment group were 3.6 times higher compared with that for non-treated cows. Milking out the clinical quarters cannot explain the increased new infections in the IMMA group. None of the cows with new clinical quarters were systemically ill, nor were the new clinical quarters cultured to confirm an IMI.

Microbial Growth During the First 3 d of Treatment

Culturing a cow with clinical mastitis after antibiotic therapy has generally been considered a worthless effort. However, the data reported here suggest that culturing after antibiotic therapy may be worthwhile and possibly beneficial in that a decision to change antibiotics can be made more quickly. Clearly, massive growth in the face of antibiotic therapy would suggest the need for a change.

Why FMO treatment did not produce better results is subject to speculation but might be due to the bacteria maintained in a log phase of growth. This theory is supported by the *Streptococci* data from Table 6 in which both FMO and FMO + IMMA groups were significantly more likely to have high growth (>80%) during the first 3 d of treatment vs. only 20% of IMMA with high growth. Additionally, the FMO group consistently had the highest percentage of cases with high growth when compared with the other treatment groups for all pathogens.

CMT Scores at 36 d

Even when a microbial cure occurred, in most cases, CMT scores tended to remain elevated for an extended period of time. Overall, only 42% of mastitic quarters returned to a CMT score of "trace" by 36 d with a range of 29% for *Klebsiella* to 78% for *E. coli*. This is similar to the Wisconsin study of clinical mastitis that reported 40% of clinical cases returned to a quarter SCC <400,000 cells/mL by 14 d (Timms and Schultz, 1984). With the exception of the no growth category, the FMO-

treated quarters consistently resulted in the lowest percentage of cures according to CMT score at 36 d. Combining all culture categories, the IMMA group was significantly more likely to have a CMT cure by 36 d than the FMO group.

Treatment Response Differences Among Coliforms

Studies of clinical mastitis therapy typically group all coliforms together. Data from the current study suggest that clinical mastitis caused by *E. coli* and *Klebsiella* may respond quite differently to various treatments. *Escherichia coli* were more likely to respond favorably to any treatment than *Klebsiella*. Nearly all *E. coli* cases were bacteriologically cured by 7 d (85%) vs. only 37% of *Klebsiella* cases. This result was similar to results of Smith et al. (1985), in which clinical and subclinical IMI caused by *Klebsiella* (34% cured by 9 d) were of significantly greater duration than *E. coli* IMI (71.2% cured by 9 d). Separate analyses for different coliform organisms should be considered in future efficacy studies.

Milk Production

Milk production was almost always lower after a mastitis event, as would be expected, but a difference was noted by treatment. Cows in the untreated group and cows in the FMO + IMMA group tended to have less milk production loss than either the FMO group or the IMMA group. Overall, milk production losses appear similar to those reported by Timms and Schultz (1984). In contrast, Van Eenennaam et al. (1995) showed no significant effect of treatment on 305-d mature equivalent milk production for mild cases of clinical mastitis.

CONCLUSIONS

Because of the small numbers within compared groups, this single herd study should be considered a pilot study. Although results are suggestive, they are not conclusive. Additional studies in different herds and with greater numbers are required to help verify the results of this study. In evaluating the efficacy of the four different therapeutic regimens for mild to moderate clinical mastitis, none appear superior for all mastitis pathogens. Intramammary amoxicillin may be beneficial for Gram-positive organisms such as *Streptococci* and coagulase-negative *Staphylococci*, but its use for Gram-negative organisms and fungal pathogens would seem inappropriate. These findings are in agreement with the results of a recent study in which commercially available antibiotic preparations were considered appropriate for *Staphylococcus* and *Streptococcus* isolates

but were considered ineffective for treatment of coliform mastitis (Bezek, 1998). Although untreated cases were needed for comparison purposes, no treatment may be an appropriate way to manage certain clinical mastitis cases. This was evident from the *E. coli* and the no growth data, in which untreated cases of mastitis cured just as rapidly or more quickly than cases in the other three treatment groups. Because there are no labeled products for fungal mastitis and because fungal mastitis cases tended to cure within 1 mo, no treatment would appear to be more appropriate than antibiotic therapy. Frequent milk-out appears to be an ineffective treatment for mild to moderate cases of clinical mastitis. Similarly, combining FMO with IMMA did not appear to improve recovery from clinical mastitis and cannot be recommended based on the data presented here.

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