Table 8. Amount of Body Condition Score Loss for Holstein dairy cows in 13 PEI dairy herds grouped by dianosis of selected diseases.

Disease		N	LOSS	SE	p
CYST[1]					
	Yes	43	0.86	.05	.61
	No	290	0.83	.02	
REPRO[2]					
	Yes	54	0.85	.05	.65
	No	279	0.83	.02	
MET[2]					
	Yes	51	0.87	.05	.37
	No	282	0.82	.02	
LAME[2]					
	Yes	29	0.88	.06	.42
	No	304	0.83	.02	
OTHER[1]					
	Yes	51	0.94	.05	.01
	No	282	0.81	.02	

[1] adjusted for HERD, LACT, and FCM305
[2] adjusted for HERD and FCM305

for cows that experienced any of the diagnosed diseases (Table 7). Cows that were diagnosed with the category "other," lost significantly more condition than cows that did not receive this diagnosis (Table 8). Of 80 cows that received the diagnosis of "other" 47 (58%) experienced dystocias. There was a tendency for cows that were diagnosed with metabolic disease to have lower BCS throughout the lactation.

Efficacy of a Broad Spectrum Antibiotic Versus Clinical and Subclinical Bovine Mastitis

Philip M. Sears, David J. Wilson, Brenda Smith, Eric R. Hallstead
Hal F. Schulte, Gary J. Bennett

Quality Milk Promotion Services
College of Veterinary Medicine
Cornell University
Ithaca, NY 14850

Abstract

Efficacy of a broad-spectrum antibiotic effective against gram-positive and gram-negative bacteria was investigated as an experimental drug for treatment of mastitis caused by *S aureus, Staph* spp., *Strep* spp., and *E. coli.* Ten commercial dairy farms with a total of 861 cows (73 Jerseys, 788 Holsteins) with various herd sizes and types of cow housing participated. 305 day mean actual milk production ranged from 11,000 to 23,600 pounds (4994 to 10,714 kg), and mean bulk tank SCC for 6 months ranged from 130,000/ml to 815,000 on the farms. Treatments used were experimental treatment, 750 mg, or control treatment, 200 mg of cloxacillin, administered by intramammary infusion in a double blind study. Criteria for cure involved repeated reculturing of milk over 28 days combined with somatic cell count data.

Clinical mastitis was detected in 119 quarters, 34 of which were removed from the study because additional treatment was required, or the cows were sold or died. 85 clinical mastisis cases remained which met the required case definition and protocol: 8 Strep ag, 23 *S aureus*, 25 *Strep* sp., 4 *Staph* sp., 11 *E coli*, 5 *Klebsiella*, and 9 Others. There were 71 cases of subclinical mastitis, 31 caused by *S aureus*, 10 caused by *Strep* sp., 29 caused by *Staph* sp., and one caused by *Klebsiella.*

Significant differences between the experimantal drug and cloxacillin in efficacy and overall cure rates for mastitis will be discussed in this presentation.

Introduction

Bovine mastitis continues to be the most costly disease in the dairy industry.[1,2] Reduction of mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus* has not been uniformly accompanied by reduction in clinical or subclinical mastitis caused by other pathogens such as coagulase-negative staphylococci (*Staph* sp.), non-agalactiae streptococci (*Strep* sp.), and *Escherichia coli.*[3,4] Antibiotic therapy for mastitis often results in poor cure rates, especially when compared to natural elimination rates without antibiotic therapy.[5,6] Increasing sensitivity and frequency of antibiotic testing in bulk milk and slaughter cow carcasses has necessitated longer withdrawal times for milk and meat following antibiotic treatment of mastitis.[7] With the risks and costs associated with antibiotic therapy, efficacy of such treatments should be critically evaluated. This study investigated the efficacy of an experimental broad-spectrum antibiotic effective against gram-positive and gram-negative bacteria, as a treatment for mastitis caused by *Strep agalactiae, Staph aureus, Strep* sp., *Staph* sp., *E coli, Klebsiella*, and Others.

Materials and Methods

Ten commercial dairy farms participated in the study from January to June, 1991. Three farms with a total of 117 cows (73 Jerseys, 44 Holsteins) one with freestall housing and 2 with tiestall housing, 305 day mean actual milk production ranging from 11,000 to 16,500 pounds (5,000 to 7500 kg), and mean bulk tank SCC for 6 months ranging from 130,000/ml to 815,000 were included in the study of subclinical mastitis. Clinical mastitis was studied in 7 herds with a total of 744 Holstein cows, 3 with tiestall or stanchion housing, 3 with freestall housing, and one with both types of housing. Milk production was between 14,500 and 23,600 pounds (6,583 to 10,715 kg), and bulk milk SCC ranged from 145,000/ml to 550,000 (Table 1). Mechanical milking systems on all 10 farms met standards for performance.

Table 1. Characteristics of dairy farms participating in study.

Lactating herd size	Breed	305 Actual Milk Prod.	6 mon. mean SCC	Housing
42	Jersey	11,200	820,000	Freestall
31	Jersey	11,000	140,000	Tiestall
44	Holstein	16,500	130,000	Tiestall
97	Holstein	21,800	583,000	Tiestall
60	Holstein	15,000	233,000	Freestall/stanchion
60	Holstein	18,400	455,000	Stanchion
145	Holstein	23,600	174,000	Freestall
179	Holstein	22,500	145,000	Freestall
50	Holstein	14,500	292,000	Stanchion
110	Holstein	21,500	239,000	Freestall

Treatments used were experimental treatment, 750 mg (E), or control treatment, 200 mg of cloxacillin (C), administered by intramammary infusion (IMM). A randomization schedule was used to assign each case number from 1 to 300 to either E or C treatment. For each case number, 3 of the appropriate infusion syringes (either E or C) were then identified only by black and white numerical labels, and packaged in ziplock bags with the same case number label. Experimenters and dairymen only knew the case number of the treatment syringes, but not which drug was used. The drug withdrawal times were different, but only the longer withdrawal time was adhered to for all cases.

Subclinical mastitis -

All lactating cows in the 3 study herds were aseptically quarter sampled for microbiological determination of subclinical mastitis status. Those with signs of clinical mastitis such as abnormal milk or swelling of the quarter, 6 or more episodes of clinical mastitis during lactation, or any disease requiring antibiotic or antiinflammatory therapy were excluded. Only those quarters positive for *S aureus, Staph* sp., *Strep* sp., or *Klebsiella* were included as potential cases in the study. After selection of cases for treatment, the farm was revisited, each case quarter was resampled in duplicate for culture and somatic cell count (SCC) determination, the next sequential case number was assigned, and treatment was initiated. Three IMM treatments every 12 hours were administered (all herds milked twice a day). No other therapy was allowed in order for a case to remain in the study. At 14 and 21 days after treatment began, all case quarters were aseptically single sampled for culture; at day 28, duplicate samples were collected. Also at day 28, a sample for SCC determination was collected.

Clinical signs or adverse reactions at any time during the 28 days after treatment were noted using a 5 point clinical severity code (Table 2).

Table 2. Severity Code for Scoring Clinical Mastitis

1. Normal milk and normal quarter.
2. Normal quarter but questionably normal milk.
3. Abnormal milk (definite clots and/or flakes) but little or no swelling in the quarter.
4. Abnormal milk and the quarter is swollen.
5. Abnormal milk, the quarter is swollen and the cow is physically ill.

Subclinical case definition -

Both pre-treatment samples were required to be positive for the same pathogen in order for definition as a subclinical mastitis case of either *S aureus, Staph* sp., *Strep* sp., or *Klebsiella*. Absence of clinical mastitis signs was also necessary.

Clinical mastitis -

All lactating cows in the 7 study herds were eligible for inclusion as cases of clinical mastitis, except those with concurrent disease requiring antibiotic or antiinflammatory therapy, toxic mastitis signs such as depression, dehydration, anorexia, cold or edematous quarter, and a history of 3 or more episodes of clinical mastitis during lactation. Quarters with signs of clinical mastitis such as abnormal milk or swelling of the quarter were aseptically sampled in duplicate for microbiological and SCC determination. Clinical signs at time of detection and throughout the study period, and adverse reactions were noted using the 5 point clinical severity code (Table 2). After sampling, the next sequential case number was assigned, and treatment with either E or C was initiated by the dairyman. Three IMM treatments every 12 hours were administered, and no other therapy was allowed in order for a case to remain in the study. Each clinically mastitic quarter was resampled for culture at 8 to 14 days after treatment (single sample), 15 to 21 days (single sample), and at 22 to 28 days (duplicate samples) for SCC and culture.

Clinical case definition -

If both pre-treatment samples were positive for the same bacteria, including *Strep agalactiae, S aureus, Strep* sp., *Staph* sp., *E coli, Klebsiella*, and Others (*Pseudomonas,* Yeast, *Actinomyces pyogenes, Corynebacterium bovis,* gram-negative *Bacillus*, gram-positive *Bacillus*), clinical mastitis due to that agent was diagnosed.

Treatment evaluation -

Four different criteria for definition of a cure were used. Criteria I: Culture results only from day 1 and day 28 were used. Both day 28 samples were required to be negative to be counted as a cure. Criteria II: All culture results from days 1, 14, 21, and 28 were used. All samples after day 1 were required to be negative for cure for *Staph aureus* cases. For all other agents, if days 14, 21, and one of the day 28 samples were negative (only one of the two day 28 samples was positive), this was considered a cure. Criteria III: Culture results from day 1 and day 28 only, together with SCC were used. If both day 28 samples were negative, case was defined as cured. If both day 28 samples were positive, case was defined as failure of treatment. If one of the two day 28 samples was positive, case was considered cured if day 28 SCC < 300,000/ml. Criteria IV: All culture results from days 1, 14, 21, and 28 were used, together with SCC results. If all samples after day 1 were negative, case was defined as cured. If only one of the culture samples collected after day 1 was positive, the case was considered cured if the day 28 SCC was < 300,000/ml. If two or more of the culture samples collected after day 1 were positive, the case was defined as failure of treatment.

Microbiology -

All milk samples were returned to the laboratory. Samples for SCC determination were delivered to the Dairy Herd Improvement Association laboratory where Fossomatic analysis was performed. Milk samples for bacteriological culture were plated by streaking 0.1 ml onto trypticase soy agar with 5% sheep blood and .1% esculin (TBA) (Crane Laboratories, Syracuse, NY). Plates were incubated at 37 C for 24 to 48 hours.

Preliminary microbiologic analysis used colony morphology and gram staining. Gram negative organisms were further identified by colony morphology on MacConkey agar and biochemical reactions in MIL, Urea, V.P., Simmons Citrate, adonitol and raffinose tubed medias. Streptococci were identified by hemolytic patterns, presence or absence of esculin hydrolysis and the CAMP test. Staphylococci were further identified using hemolytic patterns on blood agar and the coagulase test.

All milk samples reported as negative to culture on the initial plating were then incubated for 6 hours at 37 C. A 0.01 ml loop of the incubated milk was streaked on TBA, incubated overnight at 37 C and observed for growth. All colonies were identified using the same procedures described earlier.

Statistical analysis -

Differences in overall cure rates and for each agent of mastitis among experimental drug and cloxacillin were tested using Chi-square.

Results

There were 71 cases of subclinical mastitis, 31 caused by *S aureus*, 10 caused by *Strep* sp., 29 caused by *Staph* sp., and one caused by *Klebsiella.*

Clinical mastitis was detected in 119 quarters, 34 of which were removed from the study because additional treatment was required, or the cows were sold or died. 85 clinical mastitis cases met the required case definition and protocol: 8 *Strep ag*, 23 *S aureus*, 25 *Strep* sp., 4 *Staph* sp., 11 *E coli*, 5 *Klebsiella*, and 9 Others.

Mean clinical severity score for clinical cases at onset was 3.3, corresponding to abnormal milk but little or no swelling in the quarter (Table 2).

Differences among analysis using different criteria for judging cure rates were not significant; very few cases were affected by which criteria were used. Cure rates using Criteria IV, all culture results plus SCC results, will be addressed in the remainder of this report.

There were no significant differences among experimental drug and cloxacillin in efficacy versus bovine mastitis (Chi-square). This was true for all cases, clinical cases, subclinical cases, and for each etiologic agent (Tables 3-5).

Overall cure rates for mastitis were: *Strep ag* 5/8 (63%), *S aureus* 5/54 (9%), *Strep* sp. 16/35 (46%), *Staph* sp. 7/33 (21%), *E coli* 5/11 (46%), *Klebsiella* 3/6 (50%), Others, 1/9 (11%), all cases 42/156 (27%) (Table 3).

Cure rates for clinical mastitis were: *Strep ag* 5/8 (63%), *S aureus* 4/23 (17%), *Strep* sp. 7/25 (28%), *Staph* sp. 3/4 (75%), *E coli* 5/11 (46%), *Klebsiella* 2/5 (40%), Others, 1/9 (11%), all clinical cases 27/85 (32%) (Table 4).

Cure rates for subclinical mastitis were: *S aureus* 1/31 (3%), *Strep* sp. 9/10 (90%), *Staph* sp. 4/29 (14%), *Klebsiella* 1/1 (100%), all subclinical cases 15/71 (21%) (Table 5).

Discussion

The experimental antibiotic did not offer any advantage over cloxacillin in efficacy versus bovine mastitis.

Overall cure rates for pathogens other than *Strep ag* were low, ranging from 9% for *Staph aureus* to 50% for Klebsiella.

34 of 119 clinical cases were eliminated from study completion because other therapy was judged necessary or they died. Therefore, results of the clinical mastitis

study may provide an upwardly biased estimate of treatment success for clinical mastitis, which was still only 32%.

For *Staph* sp. cases, clinical mastitis cure rate was high but subclinical mastitis was quite resistant to therapy. For *Strep* sp. cases, clinical mastitis was poorly responsive but subclinical cases responded well. Reasons for this are not readily apparent. Perhaps chronic insidious progression of subclinical *Staph* sp. infection results in inflammatory changes and makes elimination difficult, while severity of tissue damage and structural changes is worse in acute clinical episodes with *Strep* sp.

In the dairy industry there is increasing concern about risk of antibiotic residue in milk and dairy beef. An experimental drug reputed to be effective against mastitis and a widely used, accepted control drug both showed poor efficacy versus bovine mastitis in this investigation. This lends support to the trend that dairy producers and their veterinarians increasingly consider antiinflammatory therapy and other alternatives to antibiotic treatment of bovine mastitis[8] caused by agents other than *Strep ag.*

Acknowledgements

The authors wish to thank the participating dairy producers for their help with this project.

References

1. Miller GY, Dorn CR. Costs of dairy cattle diseases to producers in Ohio. *Prev Vet Med* 1990;8:171-182. 2. Blosser TH. Economic losses from and the national research program on mastitis in the United States. *J Dairy Sci* 1979;62:119-127. 3. Erskine RJ, Eberhart RJ, Hutchinson LJ, *et al.* Incidence and types of clinical mastitis in dairy herds with high and low somatic cell counts. *J Am Vet Med Assoc* 1988;192:761-765. 4. Gonzalez RN, Jasper DE, Kronlund NC, *et al.* Clinical mastitis in two California dairy herds participating in contagious mastitis control programs. *J Dairy Sci* 1990;73:648-660. 5. Craven N. Efficacy and financial value of antibiotic treatment of bovine clinical mastitis during lactation - a review. *Br Vet J* 1987;143:410-422. 6. Erskine RJ, Wilson RC, Riddel MG. The pharmacokinetics and efficacy of intramammary gentamicin for the treatment of coliform mastitis, in *Proceedings. Intl Mast Symp* 1990;256-260. 7. Jones GM, Bishop JR, Whittier WD. On-farm tests for drug residues in milk and dairy beef. *Va Coop Ext Publ* 404-401. 8. Kopcha M, Kaneene JB, Shea ME, *et al.* Use of nonsteroidal anti-inflammatory drugs in food animal practice. *J Am Vet Med Assoc* 1992;201:1868-1872.

Table 3. Cure rate (%) by etiologic agent and drug for mastitis cases (criteria IV)

	All Cases Agent							
Drug	Strep ag	S aureus	Strep sp	Staph sp	E coli	Klebsiella	Others	All agents
E[a]	2/3(67%)	2/25(8%)	8/15(53%)	4/19(21%)	4/8(50%)	3/6(50%)	1/4(25%)	24/80(30%)
C[b]	3/5(60%)	3/29(10%)	8/20(40%)	3/14(21%)	1/3(33%)	No cases	0/5(0%)	18/76(24%)
All treatments[c]	5/8(63%)	5/54(9%)	16/35(46%)	7/33(21%)	5/11(46%)	3/6(50%)	1/9(11%)	42/156(27%)

[a] E = Experimental
[b] C = Cloxacillin
[c] All treatments = Cases treated with E or C were analyzed together for overall cure rate.

Table 4. Cure rate (%) by etiologic agent and drug for clinical mastitis cases (criteria IV).

	Clinical Mastitis Agent							
Drug	Strep ag	S aureus	Strep sp	Staph sp	E coli	Klebsiella	Others	All agents
E	2/3(67%)	2/11(18%)	4/10(40%)	2/3(67%)	4/8(50%)	2/5(40%)	1/4(25%)	17/44(39%)
C	3/5(60%)	2/12(17%)	3/15(20%)	1/1(100%)	1/3(33%)	No cases	0/5(0%)	10/41(24%)
All treatments	5/8(63%)	4/23(17%)	7/25(28%)	3/4(75%)	5/11(46%)	2/5(40%)	1/9(11%)	27/85(32%)

Please see Table 3 for explanation of abbreviations.

Table 5. Cure rate (%) by etiologic agent and drug for subclinical mastitis cases (criteria IV).

	Subclinical Mastitis Agent				
Drug	S aureus	Strep sp	Staph sp	Klebsiella	All agents
E	0/14(0%)	4/5(80%)	2/16(13%)	1/1(100%)	7/36(19%)
C	1/17(6%)	5/5(100%)	2/13(15%)	No cases	8/35(23%)
All treatments	1/31(3%)	9/10(90%)	4/29(14%)	1/1(100%)	15/71(21%)

Please see Table 3 for explanation of abbreviations.