Use of the California Mastitis Test and an On-Farm Culture System for Strategic Identification and Treatment of Fresh Cow Subclinical Intramammary Infections and Treatment of Clinical Mastitis

A.S. Leaflet R2102

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Summary and Implications

The purpose of this research was to evaluate the use of an on-farm culture system in conjunction with the California Mastitis Test for the strategic treatment of subclinical mastitis in fresh cows and clinical mastitis. CMT showed high NPV indicating it was very effective in determining uninfected quarters. However, PPV were low resulting in many CMT+ quarters being uninfected; thus many uninfected quarters being treated also. Cure rates in both the fresh and clinical animals did not change when treatment was delayed for twenty-four hours while waiting for culture results This would indicate that waiting for culture results before instituting treatment does not affect treatment success. The on-farm culture system, in conjunction with the CMT, dramatically reduced the number of animals treated with antimicrobials as compared to basing treatment The purpose of this research is to evaluate the use of an on-farm culture system in conjunction with the California Mastitis Test for the strategic treatment of subclinical mastitis in fresh cows and clinical mastitis. A cost-benefit analysis will also be used to determine the economic feasibility of an on-farm culture system for commercial use.

Materials and Methods

Fresh Cow Protocol (Figure 1): Approximately 75 animals calved between June and August at the Iowa State University Dairy Farm. Within twenty-four hours of calving, all fresh animals had a CMT performed on milk from all individual quarters. Aseptic quarter samples were also taken from all quarters and cultured for mastitis organisms using National Mastitis Council procedures. Milk from each quarter was initially swabbed on both blood and MacConkey agar plates and incubated at 37°C for twenty-four hours at the dairy. Organisms that showed growth on both the blood and MacConkey agar were assumed to be gram negative, while organisms that grew only on the blood agar were assumed to be gram positive. Plates showing growth were submitted to the clinical microbiology lab at the Iowa State University College of Veterinary Medicine for identification and antibiotic susceptibility testing. Quarters and cows testing negative on the CMT received no other treatments. Cows that were CMT positive were randomly assigned to one of two treatment groups – blocked by first and other lactations. The first group (control) was immediately treated with the antimicrobial Pirlimycin hydrocholoride (Pirsue®, Pfizer, Inc) for two total treatments twenty-four hours apart in all CMT positive quarters. The second group (wait) was not treated until culture results became available twenty-four hours post CMT. Quarters with gram negative organisms or showing no growth were not treated. All quarters that cultured gram positive organisms were treated similar to the control group. All treated animals were tested and showed negative for milk antibiotic residue screening tests before their milk was allowed into the bulk tank for commercial purposes. Aseptic quarter milk samples of all animals were tested again at approximately fourteen and twenty-eight days postcalving to evaluate infection dynamics, spontaneous cures, treatment success, and new infections. Any cows with quarters showing clinical mastitis were not used for this portion of the study.

Clinical Mastitis Protocol: Animals that developed clinical mastitis at any point during lactation (including at calving) were used in this trial. Clinical mastitis cows were identified by the milkers, and flagged for later evaluations. A CMT was performed and aseptic milk samples were taken from all CMT positive and/or clinical quarters. Samples were cultured similarly to the Fresh Cow Study. Mastitis cases were graded as follows: Grade 1 – visual abnormalities in milk only; Grade 2 – local gland abnormalities along with milk abnormalities; and Grade 3 – systemic signs or abnormalities in addition to milk and udder abnormalities. All grade 3 mastitis cases were treated symptomatically based on treatment protocols and recommendations of the ISU herd veterinarian, and were not included in the study. Quarters with Grade 1 or 2 mastitis were randomly assigned to one of two treatment groups. Quarters in the control group were treated immediately following initial evaluation using the appropriate antimicrobial (see fresh cow protocol). Quarters in the second wait group were not treated until culture results were available twenty-four hours post evaluation, with the treatment regime being similar to the fresh cow protocol. See figure 2. Other bacteriological analysis and data capture were similar to the fresh cow group.

CMT and culture data at calving was analyzed using a chi-square procedure. Sensitivity, specificity, positive predictive value, and negative predictive values were generated for the CMT at calving as well. Cure rates for

each group were also determined, along with new infection rate. A cure was considered to be achieved when a quarter that was culture positive at calving was culture negative upon subsequent testing fourteen days later. Likewise a new infection was defined as a quarter that had been culture negative at the previous test was now culture positive. Cost-benefit analysis was also performed for each group. Costs for therapy included antimicrobials, labor, and milk discard. The cost of culturing an animal was determined using labor and material costs.

Results

Results for the fresh cow trial are shown in Tables 1-3. Table 1 shows a summary of fresh animals and infection status by sample dates. A total of 66 animals that calved had complete data (46 cows, 20 heifers) with 28.3 and 70% of animals, and 8.9 and 34.2% of quarters infected at calving in cows and heifers, respectively. Predominant organism in both groups was CNS and infection percentages were reduced into lactation as a result of therapy as well as spontaneous cure.

CMT sensitivity, specificity, and predictive values (PPV and NPV) for cows and heifers for each sample date are shown in table 2. Overall sensitivity and PPV were low for both cows and heifers indicating the CMT made not be very good at identifying truly infected animals. Both high specificity and, especially, NPV substantiate that the CMT is an excellent tool to identify non-infected quarters and animals. Results were reasonably similar between heifers and cows, but heifers seemed to have a stronger CMT reaction when infected with CNS as compared to cows.

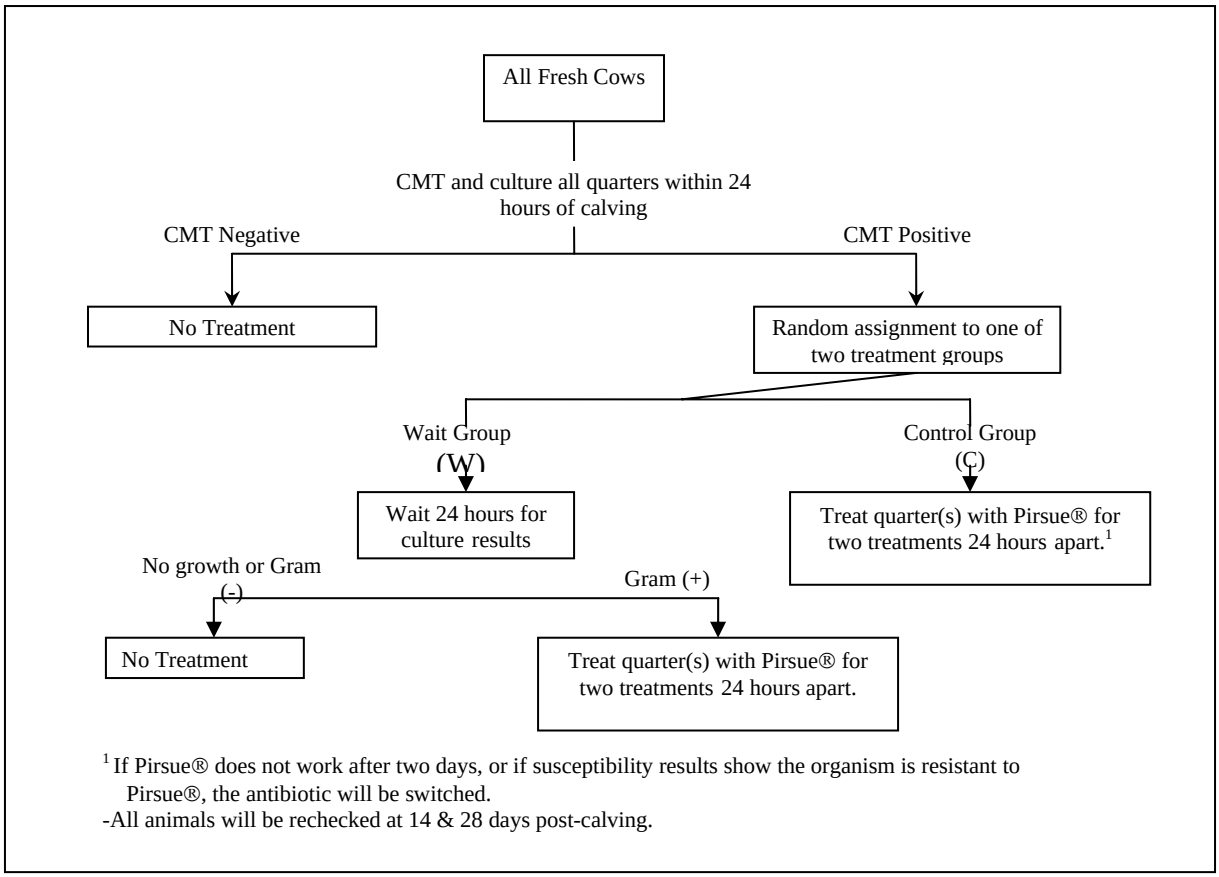
Treatment results for the fresh cow trial are shown in table 3. Overall, a high percentage of CMT positive quarters were culture negative, resulting in a high percentage of uninfected control quarters being treated. Cure rates for treated infected quarters did not different between control and wait group, or heifer and cow groups.

Results for the clinical mastitis trial are shown in tables 4-5. Table 4 shows a summary of clinical mastitis animals and infection status by sample dates. Most clinical mastitis was associated with environmental pathogens (streps. and gram negatives) or no growth. Clinical mastitis treatment results are shown in table 5. Over 50% of clinical cases showed no growth and resulted in a high percentage of these being treated in the control group. There were no differences in cure rates between groups.

A partial budget with costs and assumptions for both the fresh cow and clinical mastitis trials is shown in Table 6. Costs associated with treating fresh cows were similar between control (treat immediately) and wait (24 hour, culture) groups. Although the control group treated more uninfected cows, the wait group incurred milk discard costs as the 24 hour lag resulted in dumping saleable milk. Waiting 24 hours to treat and culturing resulted in much lower overall costs compared to treat immediately for clinical mastitis cows, primarily as a result of the high percentage of clinical cows that were culture negative.

Cure rates in both the fresh and clinical animals did not change when treatment was delayed for twenty-four hours while waiting for culture results (tables 3 and 5). This would indicate that waiting for culture results before instituting treatment does not affect treatment success. The on-farm culture system, in conjunction with the CMT, dramatically reduced the number of animals treated with antimicrobials as compared to basing treatment decisions off of the CMT or clinical symptoms only. In fresh animals the cost of waiting to treat animals and treating animals immediately was nearly equal. Although fewer animals were treated in the wait group, the cost of culturing and additional dump milk off-set the savings from treating fewer animals. However, this could change greatly based on labor and milk price and this does not take into account a reduction in potential antimicrobial residue risks. In clinical animals there was immense savings in culturing animals and only treating those necessary. Clinical situations that do not occur at calving are different than fresh cow mastitis because the amount of milk dumped during treatment is significantly greater, and the amount of milk dumped is the same regardless of when treatment is instituted. Since this is the case, spending a relatively small amount of money to perform a culture can result in larger savings because fewer animals are treated. The difference in the number of animals treated is enough that the reduced dump milk far offsets the cost of the culture.

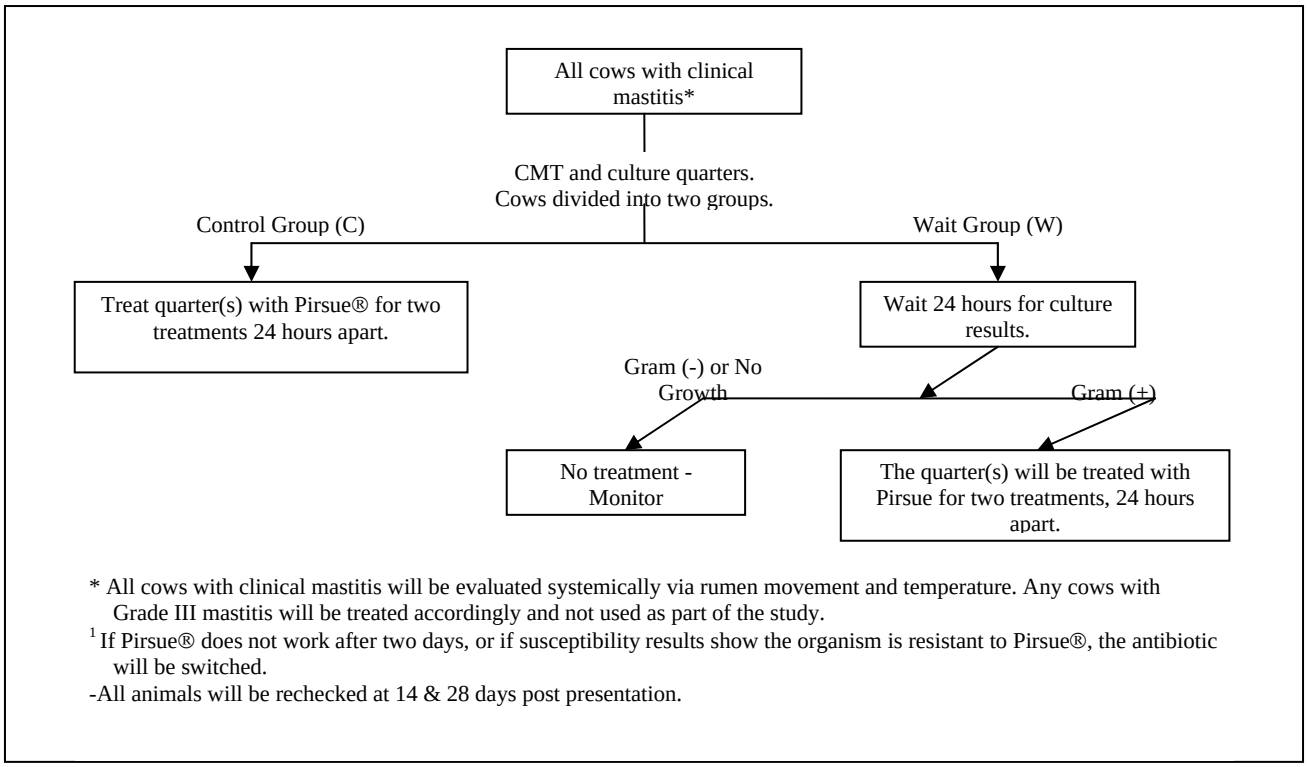
Figure 1. Fresh cow CMT, culture, and treatment protocols.



[1] If Pirsue® does not work after two days, or if susceptibility results show the organism is resistant to Pirsue®, the antibiotic will be switched.

-All animals will be rechecked at 14 & 28 days post-calving.

Figure 2. Clinical mastitis CMT, culture, and treatment protocols.



* All cows with clinical mastitis will be evaluated systemically via rumen movement and temperature. Any cows with Grade III mastitis will be treated accordingly and not used as part of the study.
[1] If Pirsue® does not work after two days, or if susceptibility results show the organism is resistant to Pirsue®, the antibiotic will be switched.
-All animals will be rechecked at 14 & 28 days post presentation.

Table 1. Summary of fresh animals and infection status by sample dates.

Summary of Fresh Animal Infections by Sample						
	Calving		2 Weeks Post-Calving		4 Weeks Post-Calving	
	Cows	Heifers	Cows	Heifers	Cows	Heifers
Number of Animals	46	20	44	20	37	16
% Animals Infected	28.3%	70%	25.0%	55.0%	19%	43.8%
Number of Quarters	180	79	171	79	140	62
Number (%) of Quarters Infected	19 (8.9)	27 (34.2)	12 (7.0)	17 (21.5)	7 (5.0)	11 (17.7)
Coagulase Negative *Staphylococcus sp.*	9	19	9	14	4	10
Coagulase Positive *Staphylococcus sp.*	2	0	1	0	1	0
Streptococcus sp.	6	6	1	0	1	0
Other Gram Positives	0	1	1	1	1	1
Gram Negative Species	2	1	0	2	0	0

Table 2. CMT sensitivity, specificity, and predictive values (PPV and NPV) for cows and heifers for each sample date.

Sensitivity & Specificity of Fresh Cow CMT				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Calving Day	63[1](90[2])%	93(93)%	52(45)%	96(99)%
14 Days Post-Calve	50(67)%	95(95)%	43(20)%	94(99)%
28 Days Post-Calve	86(100)%	95(95)%	46(30)%	99(100)%
Sensitivity & Specificity of Fresh Heifer CMT				
	Sensitivity	**Specificity**	**PPV**	**NPV**
Calving Day	52(88)%	81(81)%	58(41)%	77(98)%
14 Days Post-Calve	35(67)%	95(95)%	67(40)%	86(98)%
28 Days Post-Calve	36(0)%	92(92)%	50(0)%	87(98)%

[1] all pathogens considered. [2] all pathogens with the exception of CNS.

Table 3. Treatment results for the fresh cow trial.

Treatment Results of Fresh Animal Infections						
	Cows			**Heifers**		
	Control (Tx'd)[1]	**Wait w/ Tx[2]**	**Wait No Tx[3]**	**Control (Tx'd)**	**Wait w/ Tx**	**Wait No Tx**
Number of Animals	8	3	5	5	1	5
Number of Quarters	13	4	6	8	3	10
Culture Negative Quarters	5	-	6[4]	-	-	7
Quarters w/ CNS	1	1	1	5	-	2
CNS Quarters Cured	1	1	1	2	-	1
Quarters w/ CPS	2	-	-	-	-	-
CPS Quarters Cured	-	-	-	-	-	-
Quarters w/ *Strep.* sp.	4	1	-	3	3	-
***Strep.* sp. Quarters Cured**	4	1	-	3	3	-
Quarters w/ Gram Negative	1	-	1[5]	-	-	1
Gram Negative Organisms Cured	1	-	1[5]	-	-	1

[1] Control (Tx'd) indicates animals in the control group - all CMT positive quarters were treated.
[2] Wait w/ Tx indicates animals that were in the wait 24 hours group, and received treatement.
[3] Wait No Tx indicates animals that were in wait 24 hr group, were culture negative, and did not receive treatment.
[4] One of these quarters was inadvertently treated, however, it should not have been.
[5] This animal was also inadvertently treated, her quarter cultured a gram negative organism, and it is was assumed that she would have cleared it on her own like the other without treatment.

Table 4. Summary of clinical mastitis animals and infection status by sample dates.

Summary of Clinical Infections by Sample						
	Initial Clinical Presentation		2 Weeks Post-Presentation		4 Weeks Post-Presentation	
	Cows	Heifers	Cows	Heifers	Cows	Heifers
Number of Animals	11	7	10	7	11	6
Number of Quarters	43	28	38	28	41	23
Number (%) Clinical Mastitis Quarters	19 (44.2)	11 (39.3)	-	-	-	-
Number (%) of Quarters Infected	7 (16.3)	7 (25.0)	7 (18.4)	8 (28.6)	6 (14.6)	5 (21.7)
Coagulase Negative *Staphylococcus sp.*	4	1	4	5	6	3
Coagulase Positive *Staphylococcus sp.*	0	0	0	0	0	0
Streptococcus sp.	1	4	3	2	0	0
Other Gram Positives	0	0	0	0	0	0
Gram Negatives	2	2	1	1	0	2

Table 5. Clinical mastitis treatment results.

Treatment Results of Clinical Animal Infections						
	Cows			Heifers		
	Control (Tx'd)	Wait w/ Tx	Wait No Tx	Control (Tx'd)	Wait w/ Tx	Wait No Tx
Number of Animals	7	1	3	5	2	-
Number of Quarters	11	2	4	8	3	-
Culture Negative Quarters	6	2	4	4	1	-
Quarters w/ CNS	2	-	-	1	-	-
CNS Quarters Cured	1	-	-	1	-	-
Quarters w/ CPS	-	-	-	-	-	-
CPS Quarters Cured	-	-	-	-	-	-
Quarters w/ *Streps* sp.	3	-	-	2	2	-
***Streps* sp. Quarters Cured**	2	-	-	2	1	-
Quarters w/ Gram egative	-	-	-	1	-	-
Gram NegativesCured	-	-	-	1	-	-

[1] There are more control than wait animals because several wait group cows could not finish the study for various reasons.

Table 6. Partial budgets with costs and assumptions for both the fresh cow and clinical mastitis trials.

Partial Budget for Control & Wait Groups				
	Fresh Control	**Fresh Wait**	**Clinical Control**	**Clinical Wait**
CMT	1.00	1.00	-	-
Culture[1]	-	3.75	-	3.75
Antimicrobial[2]	9.50[3]	8.00[4]	8.00[5]	7.50[6]
Additional Dump Milk[7]	-	7.50	30.00	30.00
Total per Animal	10.50	20.25	38.00	41.25
Total Cost per 100 Animals	$199.50[8]	$202.50[9]	$285.00[10]	$99.00[11]

1 Cultures were assumed to be done in the parlor during milking time. Labor was assumed to be $10.00/hr, with a culture taking 10 minutes of total time. The rest of the cost is materials.
2 The antimicrobial was two tubes of Pirsue®, which cost $5.00.
3 In the fresh groups, on average, each treated animal had 1.9CMT (+) quarters (1.8x5).
4 In the fresh wait group, on average, each infected animal had 1.6 infected quarters (1.6x5).
5 In the clinical control group, on average, each treated animal had 1.6 clinical quarters (1.6x5).
6 In the clinical wait group, on average, each infected animal had 1.5 infected quarters (1.5x5).
7 Milk from all fresh animals is dumped for the first six milkings, only milk dumped beyond that was considered in the cost. For fresh cows, daily production was assumed to be 50 lbs/day, with milk being dumped for one extra day. For clinical cows, daily production was assumed to be the herd average of 80 lbs/day, with 2.5 days of milk being dumped. Milk was assumed to be $15/cwt.
8 According to this studies results, 9% of quarters were CMT positive at calving, therefore, 400 quarters * 9% * 1/1.9 cow/quarters = ~19 animals treated.
9 According to this study's results, only 5% of quarters were bacteriologically positive, therefore, 400 quarters * 5% * 1/1.6 cow/quarters = ~10 animals treated.
10 Approximately 3% of the herd showed clinical mastitis—all of which were treated in the control group, therefore, 400 quarters *3% *1/1.6 cow/quarter = 7.5 animals.
11 Approximately 3% of the herd showed clinical mastitis — but only ~30% were bacteriologically positive, therefore, 400 quarters * 3% * 30% * 1/1.5 cow/quarters = 2.4 animals