



The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes

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ABSTRACT

The objective of this multi-state, multi-herd clinical trial was to evaluate the efficacy of using an on-farm culture system to guide strategic treatment decisions in cows with clinical mastitis. The study was conducted in 8 commercial dairy farms ranging in size from 144 to 1,795 cows from Minnesota, Wisconsin, and Ontario, Canada. A total of 422 cows affected with mild or moderate clinical mastitis in 449 quarters were randomly assigned to either (1) a positive-control treatment program or (2) an on-farm, culture-based treatment program. Quarter cases assigned to the positive-control group received immediate on-label intramammary treatment with cephapirin sodium. Quarters assigned to the culture-based treatment program were cultured on-farm and treated with cephapirin sodium after 18 to 24 h of incubation if they had gram-positive growth or a mixed infection. Quarters with gram-negative or no growth did not receive intramammary therapy. The proportion of quarter cases assigned to positive-control and culture-based treatments that received intramammary antibiotic therapy because of study assignment was 100 and 44%, respectively; the proportion of cases that received secondary antibiotic therapy was 36 and 19%, respectively; and the proportion of cases that received intramammary antibiotic therapy because of study assignment or secondary therapy was 100 and 51%, respectively. A tendency existed for a decrease in the number of days in which milk was discarded from cows assigned to the culture-based treatment program versus cows assigned to the positive-control group (5.9 vs. 5.2 d). No statistically significant differences existed between cases assigned to the positive-control and cases assigned to the culture-based treatment program in days to clinical cure (2.7 vs. 3.2 d), bacteriological cure risk within 21 d of enrollment (71 vs. 60%), new intra-

mammary infection risk within 21 d of enrollment (50 vs. 50%), and treatment failure risk (presence of infection, secondary treatment, clinical mastitis recurrence, or removal from herd within 21 d after enrollment; 81 vs. 78%). In summary, the use of an on-farm culture system to guide the strategic treatment of clinical mastitis reduced intramammary antibiotic use by half and tended to decrease milk withholding time by 1 d, without significant differences in days to clinical cure, bacteriological cure risk, new intramammary infection risk, and treatment failure risk within 21 d after the clinical mastitis event.

Key words: clinical mastitis, selective treatment, on-farm culture, bi-plate

INTRODUCTION

Mastitis has been recognized as the most frequent reason for antibiotic use in dairy cattle (Sundlof et al., 1995; Mitchell et al., 1998). In a recent study in 20 Wisconsin conventional dairies, 80% of all antimicrobial drugs used were used for treatment or prevention of mastitis (Pol and Ruegg, 2007). Interestingly, 50% of all antimicrobial drugs used were solely for treatment of clinical mastitis. Problems attributed to the use of antibiotics in animals include those of antibiotic residues and the potential for development of antibiotic resistance (Owens et al., 1997; Barton, 2000; Sol et al., 2000; Erskine et al., 2002; Makovec and Ruegg, 2003; Pitkälä et al., 2004; Pol and Ruegg, 2007).

Another concern is that the value of discarded milk following antibiotic treatment can exceed \$100 per cow in the herd per year (Bartlett et al., 1991). Thus, increased awareness exists among producers and veterinarians of treatment-related costs and the economic costs of extensive antibacterial therapy for mastitis (Erskine et al., 2003). However, contrary to the expected decrease in discarded milk in a non-antibiotic treatment regimen, a clinical trial evaluating 2 antibiotic treatment regimens and 1 based just on the administration of oxytocin found that the cost of treatment, calculated

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by adding the cost of the therapy to the value of the milk withheld, did not differ between 1 of the antibiotic treatments and the non-antibiotic regimen. The oxytocin treatment costs were not significantly lower than for amoxicillin because of the increased number of milkings required for some of the affected quarters of the oxytocin-treated group to produce milk with a normal appearance (Van Eenennaam et al., 1995).

It has been reported that 10 to 40% of cultures from clinical mastitis cases yield no bacterial growth, and so do not require antimicrobial therapy (Roberson, 2003). Another 40% of positive cultures (gram-negatives, yeast) are not susceptible to most approved intramammary antimicrobial drugs. Also, a large proportion of gram-negative infections are quickly cleared by the cow's own immune system (although occasional persistence of gram-negative infections occurs; Erskine et al., 1992; Pyörälä et al., 1994). Conversely, intramammary antibiotic therapy is routinely recommended for infections caused by gram-positive organisms such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and environmental streptococci species. Based on these numbers, Roberson (2003) estimated that antibiotics labeled for intramammary use would not be justified for 50 to 80% of clinical mastitis cases.

Consequently, clinical mastitis treatment decisions should be based on culture results. However, laboratory culture has not been routinely used by many dairies because of the time delay between submission of milk samples and reporting of results. Adoption of rapid on-farm milk culture systems would allow producers to make strategic treatment decisions for clinical mastitis cases, based on knowing the pathogen involved. The Minnesota Easy Culture System (University of Minnesota, Saint Paul), a commercial on-farm milk culture system, offers 2 different types of selective culture media systems. The bi-plate system is a plate with 2 different types of agar: MacConkey agar on one half selectively grows gram-negative bacteria, whereas Factor agar, similar to KLMB agar (Beatty et al., 1985), on the other half of the plate, selectively grows gram-positive organisms while inhibiting the growth of gram-negative bacteria with antibiotics (University of Minnesota, St. Paul). Alternately, the tri-plate system is a plate with 3 different types of agar. In addition to including MacConkey agar and Factor agar, it also includes a section of MTKT agar, which is selective for streptococci. The bi-plate culture system results, in agreement beyond chance with laboratory results, test characteristics, and predictive values for identification of gram-positive bacterial growth (vs. no growth and gram-negative growth), were described elsewhere (Lago, 2009). Kappa values of 61%, sensitivity of 78%, specificity of 83%, predictive value of a positive result

of 74%, and predictive value of a negative result of 86% were obtained when the bi-plate was used on-farm to culture quarter secretion samples from clinical mastitis cases. The authors concluded that the Minnesota Easy Culture Bi-Plate System is a useful cow-side test to correctly identify gram-positive bacterial growth in quarter secretion samples from clinical mastitis cases. The use of on-farm milk culture for the selective treatment of clinical mastitis may represent a tremendous opportunity to decrease antimicrobial use on commercial dairy farms without sacrificing the efficacy of treatment or the long-term health and production potential of the cow. Benefits could include decreased economic cost of therapy, decreased risk of antimicrobial residues in milk, and a decrease in the potential risk for development of antimicrobial resistance in mastitis pathogens. However, many of these potential benefits need further evaluation to confirm and quantify the nature of these proposed benefits.

The objective of this study was to investigate the efficacy of using an on-farm milk culture system to guide strategic treatment decisions in cows with mild and moderate clinical mastitis. Outcomes evaluated included (1) risk of receiving primary intramammary antibiotic therapy because of study assignment, (2) risk of receiving secondary (or extended) intramammary antibiotic therapy, (3) days to return to visibly normal milk (days to clinical cure), (4) days of milk withheld from market (days out of the tank), (5) bacteriological cure risk within 21 d of enrollment, (6) new IMI risk within 21 d of enrollment, and (7) treatment failure risk (presence of infection, secondary treatment, clinical mastitis recurrence, or removal from herd within 21 d of enrollment).

MATERIALS AND METHODS

Study Design

A randomized controlled clinical trial was conducted between June 2005 and April 2007 in 8 dairy herds. In each herd, cows were enrolled in the study during a period not longer than 6 mo. These herds (2 in Minnesota, 5 in Wisconsin, and 1 in Ontario, Canada), were a convenience sample of commercial dairy farms from the North American Great Lakes Region (Table 1). Herd size ranged from 144 to 1,795 cows, averaging 920 cows. Seven of the herds were housed in freestalls and 1 in a tie-stall housing system. Annual milk production among those herds ranged from 9,545 kg to 12,818 kg, averaging 11,123 kg. Bulk tank milk SCC ranged from 182,000 cells/mL to 535,000 cells/mL, averaging 282,500 cells/mL. Selected producers were required to maintain compliance with the study protocols and

Table 1. Herd descriptors, as well as etiology of infection and herd contribution for clinical mastitis cases enrolled in the study

Item	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F	Herd G	Herd H
State or province	MN	MN	WI	WI	WI	WI	WI	ON
Housing type	Freestall	Tie Stall						
Stall base	Mattress	Mattress	Deep bed	Deep bed	Mattress	Mattress	Deep bed	Mattress
Bedding	Shavings	Sawdust	Sand	Sand	Shavings	Shavings	Sand	Shavings
Size (cows)	1,001	299	197	1,602	1,795	568	1,754	144
RHA ¹ (kg)	10,500	10,227	12,818	12,318	9,591	12,425	11,563	9,545
SCC ² (cells/mL)	261,000	296,000	192,000	334,000	182,000	223,000	535,000	237,000
Cases ³ (%)								
No growth	47	28	31	23	35	22	24	33
Gram-negatives	15	17	19	39	34	67	43	3
Gram-positives	35	53	44	38	26	11	24	63
Mixed infection	3	2	6	0	5	0	9	1
Study contribution ⁴	28	14	4	12	27	4	5	6

¹Milk production annual rolling herd average (RHA).

²Bulk tank milk SCC.

³Etiological classification was based on laboratory culture results.

⁴Number of cases of clinical mastitis enrolled in this herd/number of cases of clinical mastitis enrolled in the study.

record keeping; have trained personnel, individual animal identification, treatment facilities, appropriate drug storage capabilities, and refrigeration and freezer capacity; participate in a DHIA testing program; and demonstrate sufficient interest in the study. On-farm culturing was done by the herdsman or other dairy employee who was trained in the study protocols, on-farm culturing procedures, and record keeping before the initiation of the study. Four of the 8 dairies participating in the study were already implementing on-farm culture before the initiation of the study.

Case Definition

Clinical mastitis was diagnosed by herd personnel if milk from 1 or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter, or generalized illness. All lactating cows in the herd were eligible for enrollment at the time of clinical mastitis occurrence when only the milk was abnormal (mild or grade 1 clinical mastitis) or when the milk and the quarter were affected (moderate or grade 2 clinical mastitis). Cows exhibiting severe or grade 3 clinical mastitis (depression, anorexia, dehydration, fever) or any cow with fewer than 3 functional quarters were ineligible for enrollment.

Enrollment Process

Cows with clinical mastitis were detected in the milking parlor by the milkers upon observation of clinical signs of mastitis (e.g., visibly abnormal milk or quarter). If the cow met the designated inclusion criteria for enrollment, herd personnel aseptically collected a

single milk sample from the affected quarter. For a first clinical mastitis episode (cow not previously enrolled in the study), eligible cows for enrollment were randomly assigned following a simple randomization schedule to either the positive-control group or culture-based treatment group by opening a pre-identified sealed opaque envelope following a sequential order. If more than 1 quarter was affected, all affected quarters were assigned to the same treatment group. For a second (or greater) clinical mastitis episode in the same cow (i.e., cow had been previously enrolled) in the same or in a different quarter, the quarter was assigned to the same treatment group as was previously assigned.

Treatment Groups

Positive-Control Group. Immediately after enrollment, the quarter milk sample that had been aseptically collected was frozen on-farm at -20°C and the affected quarter(s) were infused with 1 syringe (200 mg) of cephapirin sodium (Cefa-Lak; Fort Dodge Animal Health Inc., Fort Dodge, IA). The treatment was repeated once, 12 h after the first treatment, according to label directions. A milk-withdrawal period of 96 h and a slaughter-withdrawal period of 4 d were followed after the last treatment.

Culture-Based Treatment Group. The aseptically collected milk sample(s) from the affected quarter(s) was first cultured on-farm by trained herd personnel using the Minnesota Easy Culture System (University of Minnesota, St. Paul). This on-farm milk culture system consists of a bi-plate, which is a Petri dish with 2 different types of agar: MacConkey agar on one half that selectively grows gram-negative bacteria and Factor media on the other half of the plate that selectively

grows gram-positive bacteria. A sterile cotton swab was dipped into the milk sample and then plated onto the Factor media half of the bi-plate, redipped into the milk, and then applied to the MacConkey media half of the bi-plate. The plate was placed in an on-farm incubator and incubated at approximately 37°C for 18 to 24 h. After inoculation of the swabbed media, the quarter milk sample was frozen on-farm at -20°C. The next day, the plate was examined for bacterial growth and interpreted by herd personnel according to guidelines provided by the Minnesota Easy Culture System II user's manual (University of Minnesota Laboratory for Udder Health, 2004). If bacteria did not grow, the plate was returned to the incubator and re-read approximately 18 to 24 h later. Final results for each sample plate were recorded as (1) gram-positive, when bacteria grew only in the Factor agar media of the bi-plate; (2) gram-negative, when bacteria grew only in the MacConkey agar media of the bi-plate; (3) no growth, when bacteria did not grow on either media; or (4) mixed infection when bacteria grew on both media. The decision regarding initiation of intramammary antibiotic therapy the day after enrollment of the clinical mastitis case was based on the on-farm culture results. Quarters from which gram-positive bacteria were isolated or had a mixed infection received the same intramammary antibiotic treatment following the same procedures as cases assigned to positive-control treatment. If the on-farm milk culture result was gram-negative or no growth, the quarter did not receive intramammary therapy.

After enrollment, farm personnel recorded short-term outcomes, including the number of days the cow was treated, the number of days to return to visibly normal milk, number of days out of tank, and whether or not extended (secondary) therapy was given. Study technicians visited the study herds once per week and aseptically collected single-quarter milk samples from enrolled quarters at approximately 14 d post-enrollment (10–16 d post-enrollment) and 21 d post-enrollment (17–23 d post-enrollment). All milk samples were transported on ice to the regional mastitis culture laboratory (St. Paul, MN; Madison, WI; or Guelph, ON, Canada) and frozen at -20°C until bacteriological culture was completed. In addition, study technicians observed herd personnel performing on-farm culture procedures, recorded incubator temperature and evaluated the incubator humidity conditions, and revised the bi-plate colony growth readings done by farm personnel during the previous week (used bi-plates were kept in the refrigerator by farm personnel until technicians visited the farm once per week).

Laboratory Bacteriological Culture

Aerobic culture methodologies for frozen milk samples (enrollment d 0, 14, and 21) collected on

farms were standardized among laboratories at all 3 participating sites and performed in accordance with the National Mastitis Council guidelines (NMC, 1999). Briefly, individual quarter milk samples were thawed at room temperature. While still cold, 0.01 mL of milk was plated onto MacConkey agar plates and Factor agar plates using sterile calibrated loops. Inoculated plates were incubated at 37°C. After incubation for 18 to 24 h, all plates were observed for microbial growth. Those plates having growth were recorded and species identification was started. All plates were placed in the incubator for an additional 18 to 24 h and reevaluated for microbial growth. Colonies on MacConkey agar plates were presumptively identified based on colony morphology. Colony color was used as a means of determining if the organism on the plate was a lactose-fermenting organism. Isolates were also Gram stained to assist in organism identification. Organism identity was confirmed using the API 20E test (bioMérieux Vittek Inc., Hazelwood, MO). Colonies suspected as being staphylococci, based on colony morphology, were confirmed as staphylococci, based on catalase reaction and microscopic morphology. Organisms suspected of being *Staph. aureus* were confirmed using the tube coagulase reaction. Those organisms that were catalase positive and coagulase negative were classified as *Staphylococcus* spp. Catalase-negative streptococci were streaked onto an MTKT agar plate, which is selective for *Streptococcus* spp. only, to determine the esculin reaction and presumptive identification before organism confirmation using the API *Streptococcus* identification system (bioMérieux Vittek Inc.).

Data Analysis—Definition of Outcome Variables

Risk of Receiving Primary Intramammary Antibiotic Therapy Because of Study Assignment. All clinical mastitis cases assigned to positive-control treatment were treated with 2 infusion syringes (200 mg) of cephapirin sodium (Cefa-Lak; Fort Dodge Animal Health Inc.). However, for cases assigned to the culture-based treatment group, only quarters from which gram-positive bacteria were isolated or had a mixed infection initially received antibiotic treatment.

Risk of Receiving Secondary Intramammary Antibiotic Therapy Because of Non-Responsive Cases. Secondary (or extended) treatment was allowed in cases that did not respond to the initial treatment regimen assigned. Failure to respond was defined as (1) increasing severity (i.e., became grade 3) of the clinical mastitis case within 24 to 48 h after the initial treatment regimen was implemented (either intramammary antibiotic treatment or no treatment), or (2) failure to decrease in severity (grade) of mastitis when assessed

by herd personnel approximately 48 h after the initial treatment regimen was implemented.

Risk of Receiving Intramammary Antibiotics Because of Primary or Secondary Therapy. The overall risk of receiving intramammary antibiotic treatment was represented by a dichotomous outcome denoting no administration (0) or administration (1) of intramammary antibiotic therapy because of either study assignment or secondary treatment for each quarter case of mastitis.

Days to Clinical Cure and Days Out of the Tank. Herd personnel assessed mammary gland secretion daily after enrollment, and recorded the date and time when milk had returned to being visibly normal (no clots, no flakes). The date and time when milk was first marketed after enrollment of the clinical mastitis case was also recorded by herd personnel.

Bacteriological Cure Risk. A quarter was considered infected when 1 or 2 bacterial species were isolated from a quarter milk sample. The isolation of 2 bacterial species was considered a mixed infection. A quarter sample was considered contaminated if 3 or more bacterial species were isolated. A bacteriological cure within a quarter was defined as the presence of 1 or 2 microorganisms in the enrollment milk sample, and the absence of the same specified microorganism(s) in both d-14 and -21 milk samples.

New IMI Risk. A quarter was considered newly infected whenever a new bacterial species that was not previously present in the enrollment sample (d 0) was isolated from quarter milk samples collected either at d 14 or 21 after enrollment. Contaminated samples at enrollment or new bacteria isolated from contaminated samples collected at any of the 2 sampling times after enrollment were not included in this calculation.

Treatment Failure Risk. It was considered a treatment failure if 1 or 2 bacterial species were isolated from quarter milk samples collected either at d 14 or 21 after enrollment, if secondary treatment was administered, if the quarter experienced a clinical mastitis recurrence within 21 d after enrollment (clinical mastitis recurrence was defined as detection of a new clinical mastitis case in the same quarter at least 14 d after the enrollment of the previous case of clinical mastitis), or if the cow was removed from the herd because of culling or death within 21 d after enrollment. Treatment failure was described as a dichotomous outcome. A treatment failure value of 1 indicates that any one of the previously mentioned conditions occurred. A treatment failure value of 0 indicates that none of the conditions occurred. Analysis of the treatment failure risk was done in an attempt to eliminate potential omission bias created by not including, in the bacteriological cure analysis, cases where no bacteria were isolated from

the enrollment sample, and cases without a follow-up culture result because of clinical mastitis recurrence or because the cow was removed from the herd. In addition, the treatment failure outcome eliminates the potential confounding induced by secondary treatments or treatment of recurrent cases of clinical mastitis.

Statistical Analysis—Models and Modeling Strategy

Database summaries and plots were used for exploratory data analysis. Basic diagnostic techniques were used to evaluate normality, independence, homoscedasticity, collinearity, and linearity of variables.

Generalized Linear Mixed Models for Dichotomous Outcome Variables. Binary response variables, such as risk for secondary intramammary antibiotic therapy, risk of receiving intramammary antibiotic therapy because of study assignment or secondary treatment, quarter risk for a bacteriological cure, quarter risk for a new IMI, and quarter risk for treatment failure, were modeled as a function of treatment group and other covariates using logistic multivariable regression. The treatment effect on the risk for the listed outcome variables following the clinical mastitis case was analyzed by generalized linear mixed models using the GLIMMIX PROC of SAS version 9.1 (SAS Institute, 2003) with herd and cow included as random effects to account for the clustering of cows within herds, and of quarters within cows. This was done by specifying a random statement in the model (random herd cow). The generalized linear mixed models were specified as $\text{logit}(Y_{ijk}) = \alpha + \beta'_{1ij}\mathbf{X}_{1ij} + \beta'_{2j}\mathbf{X}_{2j} + v_i + u_{jk}$, where the subscripts i , k , and j denote the i th quarter, the j th cow, and the k th herd; Y_{ijk} = the fitted probability from the i th quarter, the j th cow, and the k th herd; α = regression intercept; \mathbf{X}_{1ij} = vector of covariates associated with quarter i of cow j ; β'_{1ij} = vector of coefficients for \mathbf{X}_{1ij} ; \mathbf{X}_{2j} = vector of cow-level exposures for cow j ; β'_{2j} = vector of coefficients for \mathbf{X}_{2j} ; v_i = residual variation between quarters; and u_{jk} = random effect reflecting clustering of quarters within cows and of cows within herds.

Covariates such as cow parity, DIM at the clinical mastitis event, previous occurrence of a clinical mastitis case in the same quarter in the present lactation, number of quarters affected with clinical mastitis in the same cow at enrollment, case severity, and etiology of infection were included in the model if it was a potential confounding variable. To determine if a covariate confounded the treatment effect on the outcome, the crude estimate of treatment group (positive control- vs. culture-based treatment) was compared with the adjusted estimate for that third variable. It

was concluded that the variable confounded the association between treatment group and outcome variable if the ratio between the difference of the crude estimate and the adjusted estimate versus the crude estimate was greater than 10%. Each variable was examined for potential confounding 1 at a time by regression. Once the confounder variables were identified, the next step was to place all confounders into a full model with 2-way interaction terms between treatment and the confounder. To simplify the model, each nonsignificant interaction term was removed 1 at a time using a backward stepwise approach, starting with the least significant interaction term and running the model again until no nonsignificant interaction terms existed in the model. With nonsignificant interaction terms removed from the model, it was determined whether covariates existed in the model that were not in an interaction term that might be a confounder. The least significant term was removed and it was evaluated to determine if this affected the treatment effect estimate, with the goal being to assess whether the variable confounded the treatment-outcome relationship. If the variable was an important confounder, it was returned to the model and other variables were assessed 1 at a time to see if they were confounders. The treatment variable was forced in the model. Once all nonsignificant interaction terms were removed, as well as covariates that did not confound the exposure-outcome relationship, this was the final model. Final significance was declared at $P < 0.05$.

Time-to-Event Models. Binary responses with a time-to-event component, such as days to clinical cure and days out of the tank, were modeled using survival analysis. The Cox proportional hazards regression method was used to test the logistic analysis explanatory variables (see previously described covariates) simultaneously for their association with time until event (PROC TPHREG). The Cox model assumes a proportional hazard constant over time. This assumption was examined by the log-cumulative hazard plot to check if the lines for both treatment groups were parallel, and tested by modeling an interaction between cohort and the follow-up time. The clustering of cows within herd was considered by specifying the Covsandwich (aggregate) option in the procedure statement and then specifying herd as the level of aggregate ($id = herd$). The Covsandwich option requests a robust sandwich estimate for the covariance matrix, which results in a robust standard error for the parameter estimates. The survival function was modeled as $\lambda_j = \lambda_0 * \exp(\beta x')$, where λ_j = hazard function (risk of the event of interest in cow j at time t , where t is the follow-up time); λ_0 = baseline hazard; $\beta x'$ = linear predictor containing a vector of covariates x , with regression coefficients β .

For days to clinical cure and days out of the tank, cows were censored when the cow was removed from the herd immediately after the clinical mastitis event or when further follow-up data was not available. The assumption of independent censoring between both treatment groups was assessed by comparing the proportion of censored cows between both treatment groups. In addition, a sensitivity analysis looking at situations of complete positive correlation (every cow censored experienced the event of interest) or negative correlation (censored cows did not experience the event of interest) between censoring and the event of interest was done. If the violation of this assumption did not dramatically alter the treatment effect estimate ($<10\%$), it was concluded that censoring did not introduce bias.

RESULTS

Descriptive Data

A total of 422 cows affected with clinical mastitis in 449 quarters were enrolled in the study. Of those, 214 cows with 229 affected quarters were assigned to positive-control treatment, and 208 cows with 220 affected quarters were assigned to culture-based treatment. Cow- and quarter-level descriptors and etiology of infection at enrollment for both study groups are shown in Table 2. The severity distribution of the clinical cases enrolled in the study was 68% for mild cases and 32% for moderate cases. Cows with severe cases of mastitis were not eligible for enrollment in this study. The parity distribution of the cows at the time of the clinical mastitis event was 33, 30, and 37% for first-, second-, and third-or-greater-parity cows, respectively. The mean and median DIM to the occurrence of a clinical mastitis case were 170 and 150 d, respectively. Cows could have 1 or more quarters affected with clinical mastitis when enrolled in the study. Ninety percent of the cows had just 1 quarter affected and the remaining 10% of cows had 2 or more quarters affected.

Bacteria were isolated from 66% of quarters with clinical mastitis at enrollment. Coliform bacteria were the most commonly isolated pathogen (24% of clinical mastitis cases), followed by non-agalactiae streptococci (14% of clinical mastitis cases), CNS (9% of clinical mastitis cases), *Staph. aureus* (7% of clinical mastitis cases), and other infections (7% of clinical mastitis cases). Among coliforms, bacteria such as *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp. represented 18, 5, and 1% of all cases, respectively. Other gram-negative bacteria such as *Pantoea* spp., *Pseudomonas* spp., and *Salmonella* spp. each represented less than 1% of all cases. *Streptococcus agalactiae* was not isolated from any of the clinical mastitis cases. The non-agalactiae

Table 2. Cow and quarter level clinical mastitis case descriptors and etiology of infection at enrollment for both study groups

Item	Positive-control group		Culture-based group	
	Cows ¹	Herds ²	Cows	Herds
Sample size				
Number of quarters enrolled	229	—	220	—
Number of cows enrolled	214	—	208	—
Severity—quarter level				
Mild (first grade)	63 (145/229)	10–95	72 (159/220)	20–100
Moderate (second grade)	37 (84/229)	5–90	28 (61/220)	0–80
Parity—cow level				
First	34 (73/214)	0–57	32 (67/208)	10–57
Second	33 (71/214)	17–50	26 (54/208)	22–50
Third+	33 (70/214)	13–83	42 (87/208)	14–75
DIM—cow level				
Mean	174 (214)	125–224	166 (208)	119–195
Median	155 (214)	117–221	143 (208)	56–238
Quarters affected—cow level				
1	88 (188/214)	75–100	93 (193/208)	80–100
2+	12 (26/214)	0–25	7 (15/208)	0–20
Previous clinical mastitis event in current lactation—cow level				
No	78 (167/214)	67–88	75 (156/208)	61–100
Yes	22 (47/214)	12–33	25 (52/208)	0–39
Etiology ³ —quarter level				
No growth	32 (71/222)	23–42	34 (74/216)	10–52
Gram-negatives	27 (60/222)	0–44	23 (50/216)	0–80
<i>Escherichia coli</i>	20 (44/222)	0–67	16 (34/216)	0–41
<i>Klebsiella</i> spp.	5 (10/222)	0–10	6 (13/216)	0–66
Gram-positives	31 (69/222)	11–63	37 (80/216)	10–63
Non-agalactiae streptococci	11 (25/222)	6–24	15 (32/216)	9–38
<i>Staphylococcus</i> spp.	7 (16/222)	0–13	10 (22/216)	0–14
<i>Staphylococcus aureus</i>	6 (13/222)	0–31	8 (17/216)	0–38
<i>Bacillus</i> spp.	5 (11/222)	0–27	3 (6/216)	0–20
Other	9 (21/222)	0–20	4 (8/216)	0–13
Mixed infection/contaminated	<1 (1/222)	0–22	2 (4/216)	0–15

¹Cow and quarter-level descriptors [% (n)]. The fraction numerator was the number of quarters or cows in each category and the denominator was the number of quarters or cows enrolled (or from which data were available).

²Herd range for the different descriptors [minimum herd (%)–maximum herd (%)].

³Etiological classification was based on laboratory culture results.

streptococci, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Enterococcus* spp., and *Aerococcus* spp. represented 5, 2, 4, and 1% of all cases, respectively. It is interesting that *Bacillus* spp. was isolated in 4% of the clinical mastitis cases. Other bacteria such as *Corynebacterium bovis* and *Arcanobacterium pyogenes* each represented less than 1% of all cases.

Effect of Clinical Mastitis Treatment Program

Risk of Receiving Primary Intramammary Antibiotic Therapy Because of Study Assignment. One hundred percent of quarter cases assigned to positive-control treatment received intramammary treatment. The proportion of quarter cases receiving intramammary antibiotic therapy as a consequence of assignment to the culture-based treatment program was 44% (Table 3). This ranged from 31 to 89% for the 8 dairy herds enrolled in the study. The etiologic agent classification distribution, based on on-farm cul-

ture for the 56% of cases assigned to the culture-based treatment group that did not receive intramammary antibiotic therapy, was 27% no growth and 29% gram-negative. For the 44% of the clinical mastitis cases that did receive intramammary antibiotic therapy, it was 39% gram-positive and 5% mixed infections. Treatment decisions based on on-farm culture agreed 81% of the time with those that would be taken if based on laboratory results. Laboratory culture results for cases assigned to the culture-based treatment group that did not receive intramammary antibiotic therapy were 45% no growth, 40% gram-negative, 14% gram-positive, and 1% mixed infections. Laboratory culture results for clinical mastitis cases that did receive intramammary antibiotic therapy were 19% no growth, 8% gram-negative, 69% gram-positive, and 4% mixed infections.

Risk of Receiving Secondary Intramammary Antibiotic Therapy Because of Non-Responsive Cases. The risk for a quarter case to receive secondary (or extended) intramammary antibiotic therapy

Table 3. Risk of receiving primary intramammary antibiotic therapy and risk of receiving primary or secondary intramammary antibiotic therapy for 2 clinical mastitis treatment programs

Item	Risk of receiving primary intramammary antibiotic therapy ¹ [% ² (n) ³]		Risk of receiving primary or secondary intramammary antibiotic therapy ⁴ [% (n)]	
	Positive-control	Culture-based	Positive-control	Culture-based
Quarter ⁵	100 (229)	44 (220)	100 (229)	51 (220)
Cow ⁶	100 (214)	44 (208)	100 (214)	51 (208)
Herd range ⁷	100	31–89	100	36–100

¹Risk of receiving intramammary antibiotic therapy because of study assignment (primary).

²The fraction numerator was the number of quarters or cows in each category and the denominator was the number of quarters or cows enrolled.

³n was the denominator of the fraction from which percentages were calculated.

⁴Risk of receiving intramammary antibiotic therapy because of study assignment (primary) or because of a severity assessment decision after enrollment (secondary).

⁵Risk of a quarter affected with clinical mastitis receiving intramammary antibiotic therapy.

⁶Risk of a cow affected with clinical mastitis receiving intramammary antibiotic therapy.

⁷Herd ranges for the risk of receiving intramammary antibiotic therapy were the same for quarters as for cows.

was lower for cases assigned to culture-based treatment than for cases assigned to positive-control treatment [model odds ratio estimate of treatment effect using positive-control as reference (OR_{PC} ; 95% CI) = 0.4 (0.3, 0.7); $P = 0.002$; Table 4]. This risk was numerically lower in cases assigned to culture-based treatment in 6 of the 8 herds enrolled in the study. No other covariates in addition to the explanatory variable of interest, treatment program, remained in the model because of confounding the treatment program effect on the risk of receiving secondary intramammary antibiotic therapy.

Secondary intramammary antibiotic therapy was administered in 36% of the cases assigned to the positive-control treatment and in 19% of the cases assigned to culture-based treatment. In cases assigned to culture-based treatment, secondary therapy was administered in 13% of the non-treated cases and in 28% of cases that received intramammary antibiotic therapy at enrollment. The overall risk of receiving secondary intramammary antibiotic therapy for both treatment programs was greater in gram-positive (30%) or gram-negative (42%) cases, as compared with no-growth cases (11%). The risk difference for secondary intramammary antibiotic therapy between cases assigned to positive-control and culture-based treatments was numerically greater for gram-negative cases (57% for cases assigned to positive-control treatment vs. 23% for cases assigned to culture-based treatment) and for no-growth cases (16% for cases assigned to positive-control treatment vs. 7% for cases assigned to culture-based treatment) than for gram-positive cases (34% for cases assigned to positive-control treatment vs. 26% for cases assigned to culture-based treatment).

Risk of Receiving Intramammary Antibiotics Because of Primary or Secondary Therapy.

The risk for a quarter case to receive intramammary antibiotic therapy because of either study assignment or secondary treatment was half for cases assigned to culture-based treatment group as compared with cases assigned to positive-control treatment. The model relative risk estimate of treatment effect using positive control as reference (RR_{PC}), 95% confidence interval, and P -value were 0.51, 0.44 to 0.58, and $P < 0.001$, respectively (Table 4). Fifty-one percent of the cases assigned to the culture-based treatment group received antibiotic therapy (44% because of study assignment and 7% because of secondary treatment of cases not treated initially with antibiotics).

Days to Clinical Cure. No significant difference existed in days to return to visibly normal milk between cows assigned to both treatment programs [positive-control hazard ratio (95% CI) = 0.8 (0.6, 1.2); $P = 0.258$; Table 4 and Figure 1]. This time was numerically shorter in cases assigned to positive-control treatment in 5 of the 8 herds enrolled in the study. The only covariate that remained in the model because of confounding the treatment program effect on days to return to visible normal milk was severity of the clinical mastitis case.

The mean days for milk to return to being visibly normal was 2.7 d for cases assigned to positive-control treatment and 3.2 d for cases assigned to culture-based treatment (Table 5). The overall days out of the tank for both clinical mastitis treatment programs for no-growth, gram-negative, and gram-positive cases were 2.8, 3.2, and 3.1 d, respectively.

Days Out of the Tank. There was a tendency for fewer days of milk withheld from the market for cows with cases assigned to culture-based treatment than for cases assigned to positive-control treatment [positive-

Table 4. Treatment effects, model fit statistics, and covariance parameters for random and fixed effects of models evaluating different outcomes¹

Item	Risk of receiving secondary therapy ²	Risk of receiving primary or secondary therapy ³	Days to clinical cure	Days out of the tank	Bacteriological cure risk	New IMI risk	Treatment failure risk ⁴
Treatment effect							
Risk ratio ⁵	OR _{PC} = 0.4	RR _{PC} = 0.5	HR _{PC} = 0.8	HR _{PC} = 1.2	OR _{PC} = 0.6	OR _{PC} = 1.0	OR _{PC} = 0.8
(95% CI)	(0.3, 0.7)	(0.4, 0.6)	(0.6, 1.2)	(0.9, 1.4)	(0.3, 1.4)	(0.6, 1.6)	(0.5, 1.4)
P-value	0.002	<0.001	0.258	0.079	0.204	0.942	0.541
Fit statistics							
–2 Log likelihood	1,982	2,526	2,502	2,680	820	1,354	1,745
Covariance parameter [coefficient (SE)]							
Herd (Random effect)	0.371 (0.284)	0.662 (0.655)	—	—	1.591 (0.858)	0.048 (0.073)	0
Cow (Random effect)	0.506 (0.330)	0.295 (0.387)	—	—	<0.001	0.068 (0.311)	0.281 (0.380)
Intercept	–1.419 (0.298)	0.390 (0.350)	—	—	–0.282 (0.947)	–0.194 (0.499)	1.265 (0.184)
Treatment (main effect)							
Culture-based	–0.811 (–0.245)	–4.677 (–0.728)	–0.214 (–0.189)	0.160 (–0.091)	–0.536 (–0.356)	0.008 (–0.232)	–0.160 (0.263)
Positive-control	Reference	Reference	Reference	Reference	Reference	Reference	Reference
No. of quarters affected (covariate)							
1	—	—	—	0.372 (0.268)	—	0.239 (0.458)	—
2+	—	—	—	Reference	—	Reference	—
Severity (covariate)							
Mild	—	—	0.225 (0.141)	—	—	—	—
Moderate	—	—	Reference	—	—	—	—
Etiology (covariate)							
Gram-negatives	—	—	—	–0.341 (0.118)	1.388 (0.870)	–0.004 (0.293)	—
Gram-positives	—	—	—	–0.536 (0.124)	0.652 (0.855)	0.133 (0.273)	—
Mixed infection contaminated	—	—	—	–0.415 (0.164)	Reference	–0.035 (0.753)	—
No growth	—	—	—	Reference	—	Reference	—

¹Covariates evaluated for potential confounding included cow parity, DIM at the clinical mastitis event, previous occurrence of a clinical mastitis case in the same quarter in the present lactation, number of quarters affected, case severity, and etiology of infection. Cow parity, DIM at the clinical mastitis event, and previous occurrence of a clinical mastitis case in the same quarter in the present lactation were not confounders for any of the outcomes evaluated.

²Risk of receiving intramammary antibiotic therapy because of study assignment (primary).

³Risk of receiving intramammary antibiotic therapy because of study assignment (primary) or because of a severity assessment decision after enrollment (secondary).

⁴Risk of infection, secondary treatment, clinical mastitis recurrence, or removal from herd within 21 d after enrollment.

⁵OR_{PC} = model odds ratio estimate of treatment effect using positive-control as reference; RR_{PC} = model relative risk estimate of treatment effect using positive control as reference; HR_{PC} = model hazard ratio estimate of treatment effect using positive control as reference.

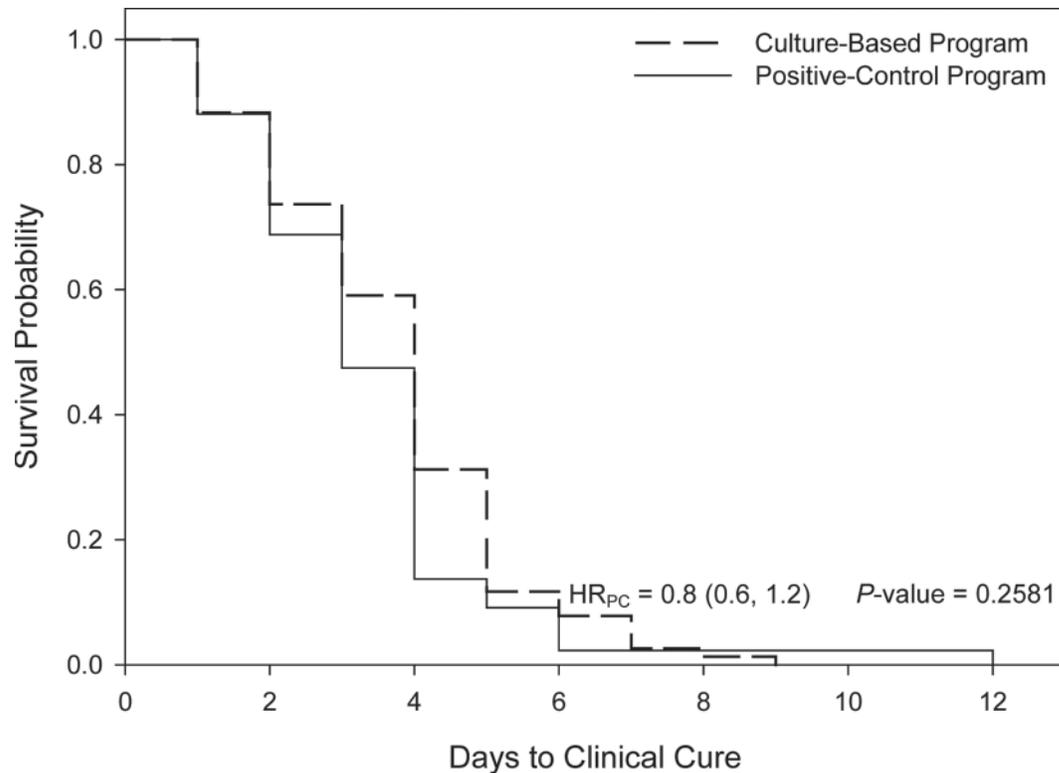


Figure 1. Kaplan-Meier survival graph representing the probability of a clinical cure at a given day after the clinical mastitis event for 2 clinical mastitis treatment programs. HR_{PC} = model hazard ratio estimate of treatment effect using positive control as reference.

control hazard ratio (95% CI) = 1.2 (0.9, 1.4); $P = 0.080$; Table 4 and Figure 2]. This time was numerically shorter in cases assigned to culture-based treatment in 5 of the 8 herds enrolled in the study. Covariates that remained in the model because of confounding the treatment program effect on days of milk withheld from the market were number of quarters affected and etiology of infection.

The average days of milk withheld from the market was 5.9 d for cases assigned to positive-control treatment and 5.2 d for cases assigned to culture-based treatment (Table 5). The difference in days of milk withheld from

the market between positive-control and culture-based treatment was much greater for gram-negative cases (6.2 vs. 4.9 d), or no-growth cases (5.5 vs. 3.9 d), than for gram-positive cases (6.1 vs. 6.5 d).

Bacteriological Cure Risk. No significant difference existed in risk for a bacteriological cure between the 2 treatment programs [OR_{PC} (95% CI) = 0.6 (0.3, 1.4); $P = 0.204$; Table 4]. The only covariate that remained in the model because of confounding the treatment program effect on bacteriological cure risk was etiology of infection.

Table 5. Days to clinical cure and days out of the tank for 2 clinical mastitis treatment programs

Etiology ¹	Days to clinical cure [mean \pm SD (n) ²]		Days out of the tank [mean \pm SD (n)]	
	Positive-control	Culture-based	Positive-control	Culture-based
No growth	2.7 \pm 1.3 (61)	3.0 \pm 1.7 (51)	5.5 \pm 2.6 (63)	3.9 \pm 3.1 (58)
Gram-negatives	3.1 \pm 2.0 (57)	3.4 \pm 1.5 (39)	6.2 \pm 2.5 (58)	4.9 \pm 2.7 (41)
Gram-positives	2.6 \pm 1.1 (54)	3.5 \pm 1.6 (62)	6.1 \pm 3.6 (62)	6.5 \pm 3.7 (72)
All cases	2.7 \pm 1.5 (196)	3.2 \pm 1.7 (163)	5.9 \pm 2.9 (183)	5.2 \pm 3.5 (184)
Herd range ³	2.2–3.5	2.6–3.8	5.0–6.3	3.6–8.3

¹Etiological classification was based on laboratory culture results.

²n is the population from which means and standard deviations were calculated.

³Herd range for the different descriptors [minimum herd (%)–maximum herd (%)].

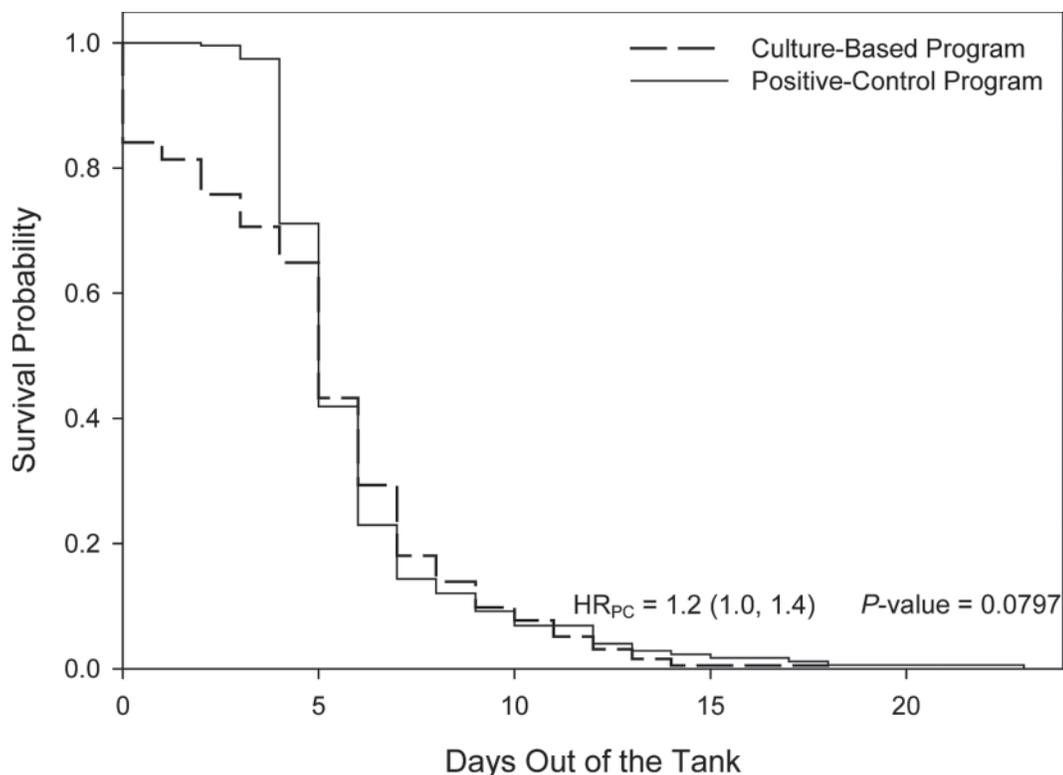


Figure 2. Kaplan-Meier survival graph representing the probability of milk to return to tank at a given day after the clinical mastitis event for 2 clinical mastitis treatment programs. HR_{PC} = model hazard ratio estimate of treatment effect using positive control as reference.

The proportion of quarters with bacteriological cure was 71 and 60% for cases assigned to positive-control and to culture-based treatment, respectively (Table 6). The overall bacteriological cure risk for both clinical mastitis treatment programs for gram-negative and gram-positive cases was 78 and 55%, respectively.

New IMI Risk. No significant difference existed in risk for new IMI between treatment programs [OR_{PC} (95% CI) = 1.0 (0.6, 1.6); $P = 0.942$; Table 4]. Covariates that remained in the model because of confounding the treatment program effect on new IMI risk included number of quarters affected and etiology of infection.

Table 6. Quarter-level bacteriological cure risk, new IMI risk, infection risk at 14 or 21 d after enrollment, and treatment failure risk (infection risk, secondary treatment, clinical recurrence, or removal from herd) within 21 d after enrollment for 2 clinical mastitis treatment programs

Etiology ¹	Bacteriological cure risk		New IMI risk [% ² (n) ³]		Infection risk (14 or 21 d)		Treatment failure risk (within 21 d)	
	Positive-control	Culture-based	Positive-control	Culture-based	Positive-control	Culture-based	Positive-control	Culture-based
No growth	—	—	50 (54)	53 (62)	50 (54)	53 (62)	64 (59)	64 (66)
Gram-negatives	86 (42)	70 (37)	52 (44)	49 (41)	63 (43)	68 (41)	89 (57)	83 (41)
<i>Escherichia coli</i>	83 (30)	78 (22)	43 (30)	41 (22)	58 (26)	57 (30)	90 (42)	77 (26)
<i>Klebsiella</i> spp.	86 (7)	62 (13)	43 (7)	55 (11)	85 (13)	63 (8)	78 (9)	92 (13)
Gram-positives	59 (46)	52 (48)	54 (54)	55 (56)	77 (56)	79 (62)	84 (64)	90 (71)
Non-agalactiae streptococci	57 (14)	61 (18)	53 (19)	63 (24)	80 (25)	79 (19)	82 (22)	93 (27)
<i>Staphylococcus</i> spp.	53 (15)	54 (13)	55 (11)	40 (10)	67 (12)	73 (11)	81 (16)	79 (19)
<i>Staphylococcus aureus</i>	43 (7)	18 (11)	14 (7)	22 (9)	82 (11)	78 (9)	83 (12)	93 (15)
<i>Bacillus</i> spp.	71 (7)	75 (4)	50 (6)	33 (3)	80 (5)	75 (8)	100 (10)	100 (5)
All cases	71 (97)	60 (85)	50 (163)	50 (160)	62 (165)	65 (168)	81 (200)	78 (181)

¹Etiological classification was based on laboratory culture results.

²The fraction numerator was the number of quarters experiencing the outcome of interest and the denominator was the number of quarters in each category.

³n is the denominator of the fraction from which the percentage was calculated.

The proportion of quarters with a new IMI was 50% for cases assigned to both clinical mastitis treatment programs (Table 6). The overall risk for new IMI for both clinical mastitis treatment programs for no-growth, gram-negative, and gram-positive cases was 52, 51, and 55%, respectively.

Treatment Failure Risk. No significant differences existed in the treatment failure risk (represents the presence of infection risk, secondary treatment, clinical mastitis recurrence risk, or removal from herd risk within 21 d after enrollment) between both treatment programs [OR_{PC} (95% CI) = 0.8 (0.5, 1.4); $P = 0.541$; Table 4]. No other covariates in addition to the explanatory variable of interest, treatment program, remained in the model because of confounding the treatment program effect on the treatment failure risk.

The treatment failure risk was 81 and 78% for cases assigned to positive-control and to culture-based treatment, respectively (Table 6). The partial contribution to the overall 79% treatment failure risk for both clinical mastitis treatment programs was 63% for the infection risk at 14 or 21 d after enrollment, 12% for the secondary treatment, 3% for the clinical mastitis recurrence risk, and 1% for the culling or death risk during this time. The treatment failure risk for no-growth, gram-negative, and gram-positive cases was 64, 87, and 87%, respectively (Table 6).

Power Analysis

The current study sample size provided an excess of 95% confidence and 80% power to detect a difference in (1) secondary intramammary antibiotic therapy of 12% or greater, (2) days to clinical cure of 1 d or more, (3) days out of the tank of 1.6 d or more, (4) bacteriological cure risk of 17% or greater, (5) new IMI risk of 16% or greater, and (6) treatment failure risk of 13% or greater.

DISCUSSION

In this study no-growth and gram-negative bacteria culture results represented 34 and 26% of all clinical mastitis cases, respectively. It has been previously reported that a shift toward environmental pathogens as the major causes of clinical mastitis in the United States, Canada, and several European Countries (Green and Bradley, 1998). In the North American Great Lakes region coliforms were recognized as a major etiology of clinical mastitis 10 and 20 yr ago (Erskine et al., 1988; Sargeant et al., 1998). However, recently, no-growth cases have become the most common culture result, as was found in the current and other studies (Wilson et al., 2004; Olde Riekerink et al., 2007).

Roberson (2003) estimated that antibiotics labeled for intramammary use would not be justified for 50 to 80% of clinical mastitis cases. The 80% estimate was based on the assumption that coliform infections and cases where bacteria were not isolated did not benefit from intramammary antibiotic therapy. In the current study, no-growth and gram-negative cases accounted for 60% of all cases. The use of the bi-plate Minnesota Easy Culture System allowed herd personnel to identify these cases and make a cow-side treatment decision the day after detection of the clinical mastitis case. As a result, only 44% of the cases assigned to culture-based treatment received intramammary antibiotic therapy as the initial treatment decision. However, because of secondary treatment decisions, the final intramammary antibiotic treatment risk for the culture-based treatment program was 51%.

The higher secondary treatment risk experienced by cases assigned to positive-control treatment, where all clinical mastitis cases were treated initially with antibiotics, may be explained in part by the possibility that herd personnel were more prone to continue antibiotic treatment, once already started, than for cases assigned to culture-based treatment where more than half of the cases were not treated initially. In cases assigned to culture-based treatment, secondary therapy was administered in 28% of cases that already received intramammary antibiotic therapy because they were identified on-farm as gram-positive or mixed infections. Similarly, secondary treatment was administered in 34% of cases assigned to positive-control treatment where gram-positive bacteria were isolated. Conversely, in cases assigned to culture-based treatment, secondary therapy was administered in only 13% of cases that did not receive intramammary antibiotic therapy at enrollment because they were identified as no-growth or gram-negative by on-farm culture. The secondary treatment risk for no-growth and gram-negative bacteria in cases assigned to positive-control treatment was 35%. Other explanations for the greater secondary treatment risk experienced by cases assigned to positive-control treatment could be the risk for contamination when infusing the antibiotic via the teat canal (Erskine et al., 2003), and possible irritation of the mammary tissue caused by the preparation. In addition, some earlier in vitro studies showed that antimicrobials may disturb phagocytosis when given intramammary (Ziv et al., 1983; Nickerson et al., 1986), but the clinical relevance of this finding is unknown.

Problems attributed to the use of antibiotics in food-producing animals include those of increased risk of antibiotic residue violations and the potential for development of antibiotic resistance. Different reports found that the majority of residue violations are related to the

antibacterial therapy of mastitis, accounting for 82 and 90% of the inhibitory residue occurrences, respectively (Reneau, 1993; Erskine et al., 2003). Furthermore, it has been established that the risk of an antimicrobial residue violation is associated with the frequency of intramammary antibiotic use on farm, and so also associated with the number of clinical mastitis cases treated with antibiotics. Farmers with an antimicrobial residue violation reported 2.01 cows treated with antibiotics per month, whereas residue-free farmers reported 1.28 cows treated per month (McEwen et al., 1991). It is expected that the on-farm culture-associated decrease in antibiotic use for the selective treatment of clinical mastitis will result in decreased antibiotic use and a decrease in the potential of antimicrobial residue occurrences. Milk processors test incoming milk for antibiotic residues and dairy farmers pay severe penalties when found to be in violation; therefore, this risk-avoidance strategy would add to the on-farm savings derived from a decrease in antibiotic use.

Appropriate use of antibiotic use in animals is a concern among animal health care providers. The Center for Veterinary Medicine of the Food and Drug Administration (FDA), in cooperation with the American Veterinary Medical Association (AVMA), compiled 15 general principles for the judicious use of antimicrobials for dairy cattle veterinarians (FDA–Center for Veterinary Medicine, 2008). These principles emphasize the use of culture to aid in the selection of antimicrobials, confine the use of antimicrobials to appropriate clinical indications, and limit therapeutic antimicrobial treatment to the fewest animals indicated. The selective treatment of clinical mastitis based on on-farm culture results implements those principles by decreasing antibiotic use by half without a decrease in treatment efficacy. If 50% of all antimicrobial drugs used in dairy farms are dedicated to clinical mastitis treatment (Pol and Ruegg, 2007), the selective treatment of clinical mastitis based on on-farm culture results has the potential to decrease total antibiotic use on dairy farms by 25%.

Discarded milk following treatment can exceed \$100 per cow in the herd per year (Bartlett et al., 1991). In the present study, a tendency existed for fewer days of milk withheld from the market for cases assigned to culture-based treatment than for cases assigned to positive-control treatment. Milk from all cows assigned to positive-control treatment required at least a 96-h milk-withdraw period because of intramammary antibiotic treatment, whereas only 51% of the cows assigned to culture-based treatment were treated with antibiotics because of primary or secondary therapy. In addition, milk from all 4 quarters was never discarded in 13% of the cases assigned to the culture-based treatment that were not treated with antibiotics due to quarter

milking the affected quarter in 2 of the 8 herds. Conversely, treatment was delayed 1 d in cases assigned to the culture-based treatment that were treated with antibiotics. The result was a tendency for an almost 1-d decrease in days out of the tank for the milk from cows assigned to the culture-based treatment. The availability of more rapid bacteria identification methods could decrease even further the amount of discarded milk. In previous studies the expected decrease in discarded milk in a non-antibiotic treatment regimen has not always been reported. A clinical trial evaluating 2 antibiotic treatment regimens and 1 based just on the administration of oxytocin found that the cost of treatment, calculated by adding the cost of the therapy to the value of the milk withheld, did not differ between 1 of the antibiotic treatments and the non-antibiotic regimen (Van Eenennaam et al., 1995). However, the authors acknowledged that the milk withhold costs for the cows in the oxytocin group included milkings during which cows were producing grossly normal milk following their recovery from mastitis, but those cows still remained in the hospital group to allow sample collection along with their contemporary antibiotic treatment group.

No significant differences existed in days to clinical cure between the clinical mastitis treatment programs. Results from previous studies are contradictory. In a clinical trial, when comparing clinical cure risk between antimicrobial and no antimicrobial clinical mastitis treatments, days to clinical cure did not differ for mild clinical mastitis cases where no bacteria (no growth), streptococci, or coliforms were isolated (Guterbock et al., 1993). Similarly, a study where cows were experimentally infected with *E. coli* and developed moderate and severe clinical mastitis, found no differences in days to clinical cure between antimicrobial and no antimicrobial clinical mastitis treatments (Leininger et al., 2003). Conversely, another study reported that when clinical mastitis was caused by *Streptococcus* spp. or coliform bacteria, the clinical cure risk by the tenth milking was significantly greater if antibiotics were used (Morin et al., 1998). The present study compares 2 different clinical mastitis treatment programs, not just antimicrobial and no antimicrobial treatment of clinical mastitis. Nevertheless, the clinical cure risk was not different for no-growth or gram-negative cases when they were assigned to the positive-control group, which included antibiotic treatment, versus those cases assigned to the culture-based treatment group, which did not include antibiotic treatment. The duration of clinical signs in the present study (mean, 3.0 d) was shorter than that reported in previous studies (5.4 and 4.1 d; Constable and Morin, 2002; Hoe and Ruegg, 2005). This difference may be attributable to the omission of severe cases

of mastitis in the present study, although differences in clinical cure or clinical mastitis case definition, cow and herd factors, as well as milker or owner awareness of prudent antibiotic usage may also explain the differences in duration of the clinical signs.

The bacteriological cure risk was not different between clinical mastitis treatment programs. Cases where coliforms were isolated had the greatest bacteriological cure, closely followed by *Bacillus* spp. A similar bacteriological cure risk existed for cases where non-agalactiae streptococci and CNS were isolated. As expected, the lowest bacteriological cure was observed for *Staph. aureus* cases. A large numerical difference existed in the risk of a bacteriological cure between both clinical mastitis treatment programs for cases where *Staph. aureus* was isolated (43 vs. 18%). This difference could be due to chance (i.e., small number of quarters represented), to the 1-d delay in initiating intramammary antibiotic treatment, or due to a failure of the on-farm culture to identify these infections. The latter reason can be discounted, as all cases assigned to culture-based treatment where *Staph. aureus* was isolated in the laboratory were identified as gram-positive and treated with antibiotics on-farm. It is not possible to discern between the 2 first hypotheses provided. However, given the fact that these cure risks are based on only 7 and 11 cases of clinical mastitis, respectively, it is very possible that these cure risk differences may be numerically different due to chance alone, and so these results should be interpreted with caution.

The infection risk and etiology of infection at 14 and 21 d after enrollment did not differ statistically between treatment groups. The infection risk at 14 and 21 d after enrollment may be a better outcome measure of both program treatment decisions than bacteriological cure risk because it represents both the bacteriological cure risk and new infection risk immediately after the clinical mastitis event, and includes in the analysis those clinical mastitis cases in which bacteria were not isolated from the enrollment sample (34% of the cases). Thus, it may more truly reflect the success of the intervention in the quarter infection status. The inclusion in the calculation of the treatment failure risk of the clinical mastitis recurrence and removal from the herd risks within 21 d after the clinical mastitis case was also done in an attempt to decrease potential omission bias of cases without a follow-up culture result because of any of those events. In addition, the treatment failure risk outcome eliminates the potential confounding induced by secondary treatments or treatment of recurrent cases of clinical mastitis. Using the treatment failure risk as a dependent variable, no differences were found between the clinical mastitis treatment groups

for the combined risk of those outcomes within 21 d after enrollment.

The omission of clinical mastitis cases where bacteria were not isolated from the enrollment sample (34% of the cases in the present study) in the bacteriological analysis could introduce selection bias due to omission of data from the analysis. In a clinical trial where treatment groups were balanced by randomization in the etiologic distribution of clinical mastitis, the quarter bacteriological status treatment effect still could be biased if the rate of new infections for cases where bacteria were not isolated differs between treatment groups. This could conceivably happen if 1 of the treatment programs had a protective effect or else introduced new infections in cases where bacteria were not isolated in the enrollment sample. It is our recommendation that future studies evaluating the efficacy of mastitis treatment programs should evaluate the treatment effect in quarter infection status during the follow-up milk sampling on cases where bacteria is not isolated. If, instead of evaluating the efficacy of a treatment program, the objective of the clinical trial is to evaluate the efficacy of an antimicrobial drug for the treatment of clinical mastitis, then the interest of the effect of treatment on bacteriological cure may be restricted to cases where bacteria are isolated before the application of antibiotic treatment (Schukken and Deluyker, 1995). Selection bias in cases without a follow-up culture result because of clinical mastitis recurrence or because the cow was removed from the herd may occur if a differential loss of follow-up occurs between treatment groups. Those losses could be due to differential management of cases assigned to the different groups or truly due to a treatment effect.

This study compared 2 clinical mastitis treatment programs that differed not just on the selective versus comprehensive usages of intramammary antibiotic for treatment of clinical mastitis. The success of the culture-based treatment program also depends on the accuracy of the on-farm culture system and the effects of a 1-d delay to initiate intramammary antibiotic therapy in those quarters selected for treatment. Previous field trials and trials with experimentally induced coliform mastitis have already reported on the inefficacy of antimicrobial treatment (Guterbock et al., 1993; Hallberg et al., 1994; Van Eenennaam et al., 1995; Leininger et al., 2003; Roberson et al., 2004). In addition, it had been reported that treatment of mild or moderate clinical mastitis cases can be postponed for 1 d with minimal adverse effects, while producers wait for on-farm culture results (Wagner et al., 2007). The present study is the first to evaluate a clinical mastitis selective treatment decision based on on-farm culture, made the

next day after the clinical mastitis event. Therefore, the strengths of this study are related to the validation of a program to treat clinical mastitis.

One potential limitation of this study is that the label of intramammary antibiotic administered in this study, Cefa-Lak (Fort Dodge Animal Health Inc.) does not include efficacy claims against gram-negative bacteria. Two intramammary antibiotic preparations, one containing hetacillin potassium equivalent to 62.5 mg of ampicillin activity (Hetacin-K; Boehringer Ingelheim USA, Ridgefield, CT) and the other containing 125 mg of ceftiofur (Spectramast LC; Pfizer Animal Health Inc., New York, NY), are currently approved in the United States with a label that claims efficacy against clinical mastitis in lactating dairy cattle associated with *E. coli*. However, published peer-reviewed studies are lacking that report the efficacy of these 2 antimicrobial formulations to treat clinical mastitis in cases where *E. coli* or other gram-negative pathogens are isolated. A need exists for controlled field trials evaluating their efficacy in treating clinical mastitis. Furthermore, clinical mastitis-selective treatment programs based on on-farm culture results using these intramammary antibiotic preparations should be evaluated. Until this scientific knowledge becomes available, the validity of the present study results when using antibiotics other than cephalosporin sodium is not known.

CONCLUSIONS

The use of an on-farm culture system to guide the strategic treatment of clinical mastitis decreased intramammary antibiotic use by half and tended to decrease withholding time by 1 d, without significant differences in days to clinical cure, bacteriological cure risk, and new infection risk or treatment failure risk within 21 d after the clinical mastitis event. Results of this study, in addition to long-term outcomes (Lago et al., 2011), will be used to evaluate the overall cost-benefit of using an on-farm culture system to guide strategic treatment decisions in cows with mild and moderate clinical mastitis.

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