

Pilot study: Impact of using a culture-guided selective dry cow therapy program targeting quarter-level treatment on udder health and antibiotic use

K. Patel¹, MVSc; S. M. Godden¹, DVM, DVSc; E. E. Royster¹, DVM, MS; J. A. Timmerman¹, BS; B. A. Crooker², MS, PhD; N. McDonald³, BS

¹Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN 55108

²Department of Animal Science, University of Minnesota, Saint Paul, MN 55108

³Herd Management Application Specialist, Boumatic, Madison, WI 53716

Corresponding author: Kruthikaben Patel; patel310@umn.edu

Abstract

The study objective was to evaluate the effects of applying a culture-guided, quarter-level selective dry cow therapy (SDCT) program on udder health and antibiotic usage as compared to blanket dry cow therapy (BDCT). Two days prior to dry-off (DO), 56 cows were randomly assigned to a BDCT or SDCT group and aseptic quarter milk samples were collected for laboratory culture. For cows in the SDCT group, milk from each quarter was also cultured using a rapid culture system, with results recorded as bacterial “growth” or “no growth”. On the day of DO, cows in the BDCT group were infused with an antibiotic and an internal teat sealant in all 4 quarters. For cows assigned to the SDCT group, quarters with bacterial growth were infused with an antibiotic plus internal teat sealant, while quarters with no growth were infused with internal teat sealant only. Quarter milk samples were collected days 1 through 7 after calving for laboratory culture, and clinical mastitis events were recorded between DO and 30 days-in-milk. Logistic regression was used to evaluate the effect of treatment on measures of udder health after calving. Results showed no effect of treatment on quarter-level risk for presence of infection after calving (42.1% vs 39.6%), cures (82.3% vs 88.0%), or new infection (41.5% vs 40.2%) for the SDCT and BDCT group, respectively. Antibiotic use was reduced by 48% in the SDCT group. Results of this pilot study suggest that a culture-guided quarter-level SDCT program can achieve equal udder health compared to BDCT, while significantly reducing antibiotic use at DO.

Key words: dairy, selective dry cow therapy, mastitis, antibiotics

Résumé

L'objectif de cette étude était d'évaluer l'effet de l'utilisation d'une thérapie sélective au tarissement (SDCT), basée sur des résultats de culture au niveau du quartier, sur

la santé du pis et sur l'utilisation d'antibiotique par rapport à une thérapie universelle au tarissement (BDCT). Deux jours avant le tarissement, on a alloué au hasard 56 vaches soit au groupe SDCT ou soit au groupe BDCT et on a recueilli des échantillons aseptiques de lait de chaque quartier pour la culture au laboratoire. Pour les vaches du groupe SDCT, le lait de chaque quartier a aussi été mis en culture avec un système de culture rapide indiquant si la croissance bactérienne était présente ou non. Au jour du tarissement, les vaches du groupe BDCT recevaient en infusion un antibiotique et un scellant à trayon intramammaire dans chacun des quartiers. Pour les vaches du groupe SDCT, les quartiers avec croissance bactérienne ont été infusés avec un antibiotique et un scellant à trayon intramammaire tandis que les quartiers sans croissance bactérienne ne recevaient que le scellant à trayons intramammaire. Des échantillons de lait de chaque quartier étaient recueillis pendant les sept premiers jours suivant le vêlage pour culture en laboratoire. L'occurrence de mammites cliniques était prise en note du jour de tarissement jusqu'au trentième jour en lait. La régression logistique a été utilisée pour évaluer l'effet du traitement sur des mesures de la santé du pis après vêlage. La comparaison des groupes SDCT et BDCT n'a pas montré d'effet du traitement au niveau du quartier sur les chances d'infection suite au vêlage (42.1% v. 39.6%), sur les chances de guérison (82.3% v. 88.0%) ou sur les chances d'observer de nouvelles infections (41.5% v. 40.2%). L'utilisation d'antibiotique a été réduite de 48% dans le groupe SDCT. Cette étude pilote suggère qu'une thérapie sélective pour vaches taries (SDCT) basée sur des résultats de culture au niveau du quartier permet d'obtenir les mêmes résultats qu'une thérapie universelle pour la santé du pis en plus de réduire significativement l'utilisation d'antibiotique au tarissement.

Introduction

Rigorous implementation of the National Mastitis Council 10-point plan to control mastitis has resulted in a dramatic

change in the incidence and etiology of bovine mastitis over past decades.⁵ The significance of environmental pathogens or minor pathogens such as coagulase-negative staphylococci and *Corynebacterium bovis* needs to be reconsidered, as in many countries they have become the most common mastitis-causing agents.^{30,48} Prevention and control of infection by these environmental organisms begins during the preceding dry period. Studies have demonstrated that the mammary gland is most susceptible to new intra-mammary infections (IMI) during the early dry period, the involution phase,²⁰ and again in the late dry period during the colostragenesis phase, and a majority of these infections are due to environmental bacteria.⁶ Smith et al demonstrated that the incidence of new IMI caused by environmental pathogens during the dry period is 10 times higher than during lactation.

The practice of blanket dry cow therapy (BDCT) is an important and widely adopted component of the dry-cow mastitis control plan, and involves infusing all quarters of all cows at dry-off (DO) with a long-acting antimicrobial (Ab) agent. This therapy is designed to eliminate preexisting infections caused by susceptible bacteria, and to prevent new IMI from developing during the early dry period. Historically, BDCT has played an important role in reducing the prevalence of contagious mastitis pathogens and thereby reducing bulk-milk somatic cell count (SCC).^{5,39} However, given steadily improving udder health in the United States, the practice of BDCT may no longer be necessary for many herds. A recent dry-cow study of midwest dairy herds reported that in some herds fewer than 20% of quarters were infected at DO.¹ Although studies to date report no indication of increasing resistance of mastitis pathogens to antimicrobials commonly used in dairy cattle,^{17,18,32} there is growing concern regarding the potential for Ab use in food animals to promote the future emergence of antimicrobial resistance in bacteria.^{10,28} The majority of Ab used on dairy herds are associated with treatment or prevention of mastitis, of which two-thirds are dry-cow products.³¹ As such, alternatives to the practice of BDCT could offer the potential to reduce Ab use at DO, provided these alternative strategies will continue to maintain or improve udder health, productivity, and animal well-being, and are cost-effective.

Selective dry-cow therapy (SDCT) is an approach whereby the decision to administer Ab treatment at DO is based on knowing the likely infection status of the cow or her individual quarters. Infected cows (or quarters) receive Ab therapy at DO, while uninfected cows (or quarters) do not. Selective dry-cow therapy programs have the potential to reduce Ab use at DO, but in order to be successful they must be equally effective for maintaining udder health as compared to BDCT, and also be cost-effective. Several early studies reported that SDCT programs were not as effective as BDCT programs because udder health of SDCT cows was reduced in the subsequent lactation.^{2,34,44,46} For example, Rindsig et al reported an increased incidence of mastitis in the selective therapy group. Likewise, Schukken et al reported increased

incidence of clinical mastitis in quarters not infused with Ab compared to quarters infused with Ab. A recent study by Scherpenzeel et al evaluated the use of SDCT in low SCC cows and made Ab treatment decisions based on somatic cell count (SCC) at the last milk recording before DO. This study reported higher SCC and an increased incidence of clinical mastitis in quarters dried-off without Ab as compared to quarters dried-off with Ab. The diagnostic sensitivity (Se) of the SCC test has been reported to range between 57.4% to 74.5%, meaning that between 42.6% to 25.5% of truly infected cows in a SCC-based SDCT program may go undetected and untreated at DO.^{41,43} Thus, early SDCT program failures may have occurred, in part, because the diagnostic tests used (e.g. SCC) were not sufficiently sensitive to identify infected cows at DO. Another potential factor contributing to failure of many early SDCT programs may have been related to having no strategy in place, such as the use of teat sealants, to protect untreated quarters from acquiring new IMI during the dry period. However, the introduction of more sensitive on-farm tests such as rapid milk culture^{a,b} and the introduction of internal teat sealants (ITS), such as Orbeseal^c to prevent new IMI during the dry period, may offer potential solutions to these challenges.

Cameron et al reported that the 3M Petrifilm rapid culture system^a had a Se of 85.2% to detect IMI at the cow-level. A more recent validation study reported a very high diagnostic sensitivity (92.6%) for the Minnesota EasyTM 4CastTM plate^b to detect IMI in individual quarters.³⁸ In a recent multi-herd randomized cow-level study, cows with a positive composite milk culture^a were assigned to the SDCT group and received Ab and ITS in all 4 quarters at DO, while cows with no growth in the composite milk sample were treated with only ITS in all 4 quarters. By comparison, all cows assigned to the BDCT group received both Ab and ITS in all 4 quarters. Results showed that adoption of the culture-guided cow-level SDCT program resulted in equal udder health in the subsequent lactation compared to BDCT, while reducing Ab usage by 21%.¹² We hypothesize that application of a culture-guided SDCT program targeting quarter-level, instead of cow-level, treatment decisions has the potential to result in equal udder health to BDCT, while reducing Ab use even further.

The major objective of this study was to complete a pilot study evaluating the effects of adopting a novel culture-guided quarter-level SDCT program on udder health and Ab usage. Secondary objectives were to describe test characteristics of the Minnesota EasyTM 4CastTM on-farm culture (OFC) system for detecting IMI in individual quarters, and to describe the economics of the SDCT program. We hypothesized that using a culture-guided SDCT program that targets individual quarter treatment decisions would result in post-calving udder health that was not inferior to BDCT. We also hypothesized that the SDCT program would result in reduced antimicrobial usage and would be cost effective, as compared to the BDCT program.

Materials and Methods

Project site and cow selection

The study was conducted at the University of Minnesota (UMN), Saint Paul campus dairy barn between July 2015 and March 2016. All project related activities were approved by the Institutional Animal Care and Use Committee (IACUC), University of Minnesota (Protocol 1506-32691A). To be eligible for inclusion, cows had to have 4 functioning quarters, have received no parenteral or intramammary treatment with an Ab or anti-inflammatory medication during the 14-day period prior to DO, be clinically healthy, and show no signs of clinical mastitis when assessed at enrollment or on the day of DO. Cows had to have an expected dry period of 30 to 90 days in duration. Cows not meeting the aforementioned inclusion criteria were excluded from enrollment.

Cow enrollment, sampling and randomization to treatment (2 days prior to dry-off)

All enrollment and sampling activities were conducted by the same study technician. At the time of regular morning milking, cows were visually inspected for clinical signs of illness and those with signs of illness were not included in the study. The udder and milk were inspected for signs of clinical mastitis. The teats were dipped in iodine-based pre-dip and cleaned with a clean towel before the actual sample collection procedure. The teat ends were then scrubbed with gauze soaked in 70% isopropyl alcohol, 3 squirts of forestripping milk was stripped out, and milk samples (approximately 10 mL) from each quarter were collected aseptically into separate sterile vials. The quarter milk samples were placed on ice immediately and transported to the UMN Udder Health Laboratory (UHL) within 30 to 45 minutes of sample collection. Following sample collection, cows were assigned to either the BDCT or SDCT program, using the Excel RAND function^d with randomization blocked within each day of enrollment (once a week) by parity (1, 2+) and breed (crossbred vs purebred).

Laboratory microbiological culture

Fresh quarter milk samples collected from all quarters of all cows (BDCT and SDCT groups) were subjected to routine laboratory culture in the UHL using procedures as defined by standard industry guidelines for mastitis diagnosis.^e Briefly, 0.01 mL of milk was streaked across one-half of a blood agar plate and MacConkey agar. The plates were incubated in an inverted position at 98.6°F (37°C) for 36 hours. Matrix-assisted laser desorption/ionization – Time of Flight^f (MALDI – TOF) mass spectrometry⁴² was used for species identification. Quarter milk samples collected from the SDCT group were also plated onto the Minnesota Easy™ 4Cast™ plate.^b Briefly, this OFC system is a single plate partitioned into 4 quadrants and contains a non-selective media that is modified to enhance the zone of hemolysis for improved identification of *Staphylococcus aureus*. A sterile single-use disposable cotton swab was dipped into the quar-

ter milk sample and used to apply approximately 0.1 mL of milk evenly to 1 quadrant of the plate. After each of the 4 quarter milk samples were streaked onto the appropriately labeled quadrant of each plate (e.g. left hind, right hind, left front, right front), the plate was identified by cow ID, and incubated at 98.6°F (37°C) for 36 hours. On the morning of scheduled DO, the rapid culture system plates were read and each quarter classified as positive for bacterial “growth” (G) if 1 or more colonies were present (equivalent to 100 cfu/mL), or as “no growth” (NG) if no colonies were present. For the OFC system there was no definition for contaminated samples; they were identified as G. The study technician performing and interpreting the rapid-culture system results was blinded to the results of routine lab culture (gold standard test) and vice versa.

Dry-off procedures

Two days after enrollment, cows were dried off once a week after the regular morning milking. After detachment of the milking unit but before dry cow treatment, teat ends were prepared by scrubbing with gauze soaked in 70% isopropyl alcohol. For cows in the SDCT group, quarters with G using the rapid-culture system were infused with a long-acting intramammary Ab formulation containing 500 mg of ceftiofur hydrochloride,^g followed by infusion with an ITS composed of 65% bismuth subnitrate.^c Conversely, quarters that showed NG were infused solely with the ITS. For cows in the BDCT group, all quarters were infused with both the Ab and ITS. Following administration of treatment(s), all quarters of all cows were dipped with a 1% iodine-based post-milking teat disinfectant. Enrollment information recorded for each cow and quarter included cow ID, breed, quarter, parity, DO date, last DHI test SCC value, treatment group assigned (SDCT or BDCT), Minnesota Easy™ 4Cast™ plate culture results for the quarter (G/NG), treatment administered to the individual quarter (Ab + ITS; ITS alone), and in-lab (gold standard) culture results for the quarter milk samples collected at enrollment 2 days prior to DO.

Post-enrollment monitoring and sampling

Following DO, all cows were monitored through the dry and fresh period by the farm personnel. Post-calving (PC) quarter milk samples were collected aseptically from all cows between 1 and 7 DIM using the methods previously described for collection of DO samples, and were cultured in the laboratory as previously described. Post-enrollment information recorded for each cow included calving date, dry period length, any clinical mastitis event detected by farm staff between DO and 30 DIM, and death or culling events. Clinical mastitis was defined as visibly abnormal milk, with or without heat, swelling, or redness in the quarter.

Definitions

1. *Presence of an intramammary infection at dry-off or after calving.* Laboratory culture of single quarter samples

was used to establish IMI status of each quarter at DO and PC. A quarter was considered to have an IMI if ≥ 100 cfu/ml was detected for any organism, with an exception of coagulase negative staphylococci (CNS) and *Bacillus* spp. For CNS and *Bacillus* spp, a definition of ≥ 200 cfu/ml and ≥ 500 cfu/ml was used to declare presence of IMI, respectively. These definitions are in accordance with the recent publication of characterization of IMI based on single-sample bacteriological culture.¹⁶ Because no peer-reviewed studies have identified a cutoff point for *Bacillus* spp, the cut-off of ≥ 500 cfu/ml was that which was previously established during an informal discussion among mastitis experts.^h A single IMI was defined as the presence of only 1 type of bacterial species. Mixed infections corresponded to the presence of 2 different bacterial species in a sample. Samples growing 3 or more different bacterial isolates were classified as contaminated and not considered as an IMI. However, when *Staphylococcus aureus* was identified in a contaminated sample, it was identified as an IMI and the quarter classified as being infected with *S. aureus*.³⁴

2. Bacteriological cure. A quarter was considered cured if a pathogen isolated in the DO sample was not detected in the PC sample. If a quarter had a mixed infection at DO (2 pathogens), the absence of both pathogens in the PC sample was required for that quarter to be considered cured. Only quarters with an IMI at DO were eligible to experience a cure.

3. New intramammary infection. A new IMI was defined as the presence of 1 or 2 new pathogens in the PC sample that were not previously present in the DO sample. All quarters enrolled, whether infected or uninfected at DO, were eligible to experience a new IMI.

Statistical Analysis

All data analyses were performed in SAS (version 9.4).ⁱ Descriptive statistics and plots were generated for exploratory data analysis. Basic diagnostic techniques were used to evaluate for normality and presence of outliers for continuous variables (e.g. previous lactation milk yield, last DHI test linear score (LS), and dry period length) and effects of treatment were determined using a student's T-test. Cow and quarter characteristics assigned to the 2 treatment groups at DO were compared using the chi-square test for categorical variables such as breed, parity at DO, prevalence of IMI at DO, prevalence of IMI at calving, cure rate, incidence of new IMI, and incidence of clinical mastitis between DO and 30 DIM.

Effect of the selective dry cow therapy program on measures of udder health

Univariable logistic regression analysis was initially conducted to describe the relationship between each of the 4 dependent variables: 1) risk for presence of IMI at calving; 2) risk for cure during the dry period; 3) risk for acquiring a new IMI during the dry period; and 4) risk for clinical mastitis between DO and 30 DIM and possible explanatory variables

(e.g. treatment group, parity, previous lactation milk yield, last test LS, dry period length). Previous lactation milk yield, last DHI test LS prior to DO, and dry period length were offered as continuous variables. Cow parity (1 vs 2+) and breed (crossbred vs purebred) were offered as categorical variable. Variables associated with the dependent variable with $P < 0.2$ in the univariate model were subsequently offered to a multivariable mixed logistic regression model to evaluate effects of treatment group (SDCT vs BDCT) on each of the 4 dependent outcomes of interest using PROC GLIMMIX procedure. Cow was included as a random effect to account for the clustering of quarters within cows. Non-significant variables were removed 1 at a time using a backward stepwise approach, with final significance declared at $P < 0.05$. The primary predictor of interest, treatment group (SDCT vs BDCT), was forced into all models regardless of statistical significance. All possible 2-way interactions were explored between treatment group and any other remaining significant covariates.

Power analysis

A priori sample size calculations were not performed because cow numbers to be enrolled were determined by budget constraints for the study. However, post hoc power analysis was performed to investigate the effect of treatment group on all of the dependent variables.

Partial budget analysis of selective dry-cow therapy

A partial budget analysis was completed to evaluate the relative economics of adopting the SDCT program in this herd. Expenses (\$ per cow) considered in the analysis included the cost of the Minnesota Easy™ 4Cast™ plate (\$3.00 per plate = per cow), sampling supplies (gloves, milk vials, alcohol wipes, cotton swabs, and culture plates), labor (sample collection, plating milk, and interpreting OFC results), and purchase of an on-farm incubator (cost amortized over a 5 yr period). Reduced Ab used in the SDCT group was considered as savings (\$ per cow). The return was calculated as the difference between savings and expenses (\$ per cow).

Diagnostic test characteristics of the rapid culture system

Test characteristics of the Minnesota Easy™ 4Cast™ plate were calculated by comparing OFC results (G/NG) to the results of lab culture of a corresponding single quarter sample (gold standard: IMI/no IMI) using 2 x 2 table in SAS. Sensitivity (Se) was defined as the proportion of quarters with an IMI truly present that were correctly classified as G for growth on the Minnesota Easy™ 4Cast™ plate. Specificity (Sp) was defined as the proportion of quarters without an IMI that were correctly classified as NG for no growth on the Minnesota Easy™ 4Cast™ plate. The positive predictive value (PPV) was calculated as the proportion of Minnesota Easy™ 4Cast™ plate positive quarters that truly had an IMI, and the negative predictive value (NPV) was calculated as the proportion of Minnesota Easy™ 4Cast™ plate negative quarters that did not have an IMI. The kappa statistic was calculated

to describe the level of agreement beyond chance between the Minnesota Easy™ 4Cast™ plate results and laboratory bacteriological culture.¹⁵

Results

Cow and quarter characteristics at enrollment

A total of 56 cows were enrolled between July and December 2015. Of these, 28 cows (112 quarters) were assigned to the BDCT and SDCT group, respectively. Post-enrollment attrition resulted in the loss of 4 cows for the following reasons: abortion (n = 1), not pregnant (n = 2), and actual dry-period length less than 30 days (n = 1). No differences were observed between study groups with respect to post-enrollment attrition. Thus, 100 BDCT group quarters (25 cows) and 108 SDCT group quarters (27 cows) were available for final analysis. No differences were detected between the 2 treatment groups in terms of cow enrollment characteristics, and the PC sample was collected at a median of 4 days-in-milk for both treatment groups (Table 1).

Of the 208 quarters sampled for laboratory culture at DO, 13 (6.2%) were contaminated, leaving 195 quarters to describe the prevalence of IMI at DO. The overall prevalence of IMI at DO was 34.8% (SDCT = 36.9%; BDCT = 32.6%) and was not different ($P = 0.51$) between treatments (Tables 2 and 3). The pathogen most commonly isolated from milk samples at DO was CNS, representing 44.0% of all isolates recovered, followed by *Bacillus* sp (16.7%) and *Corynebacterium* sp (7.1%). Gram-positive and gram-negative organisms represented 94.0% and 6.0% of all organisms isolated at DO, respectively (Table 2). In the final model, there were no significant covariates predicting the presence of IMI at DO.

Effect of treatment on risk for presence of intramammary infection at calving

Of the 208 quarters sampled at calving, a total of 193 quarters were used for analysis of risk for the presence of infection at calving, due to omission of 15 (7.2%) PC contaminated samples. The overall proportion of quarters with an IMI at calving was 40.9% and was not different between the treatment groups (SDCT = 42.2%; BDCT = 39.6%) [odds ratio (OR_{SDCT}) = 1.12 (95% CI: 0.61, 2.05)] ($P = 0.71$, Table 3). Model covariates that contributed to predicting the presence of IMI at 1 to 7 DIM included breed, with the prevalence of infection being lower in purebreds as compared to crossbreds ($P = 0.01$) [$OR_{purebreds} = 0.45$ (95% CI: 0.25, 0.83)]. The pathogen most commonly isolated from PC milk samples was CNS (19.4% of all isolates recovered), followed by *Aerococcus* sp (21.3%) and *Bacillus* sp (13.9%). Gram-positives, gram-negatives, and “others” represented 95.4%, 2.8%, and 1.8% of all isolated organisms, respectively (Table 2).

Effect of treatment on risk for cure between dry-off and calving

A total of 68 quarters had an IMI at DO and were at risk for a cure. However, 9 of these quarters (SDCT = 4 and BDCT = 5) had a contaminated PC milk sample, and therefore could not be assigned a cure status, and were not included in the analysis. As such, 59 quarters were included in the final analysis. Overall, the proportion of quarters experiencing a cure between DO and calving was 85% with no difference between the treatment groups (SDCT = 82.3%; BDCT = 88.0%) [$OR_{SDCT} = 0.604$ (95% CI: 0.11, 3.06)] ($P = 0.53$, Table 3). There were no other significant covariates in the model predicting the risk for cure.

Table 1. Characteristics of cows enrolled in 1 herd for evaluation of SDCT versus BDCT.

Item	SDCT* (n = 27 cows)					BDCT† (n = 25 cows)				
	Number (%)	Median	Mean	Std. dev.	Range	Number (%)	Median	Mean	Std. dev.	Range
Prev. lact. milk yield (kg)		5151.0	5130.2	535.7	3856.0-5849.1		5307.1	5363.3	569.3	4436.2-6763.6
DIM‡ at post-calving sampling (days)		4.0	3.6	1.3	1-6		4.0	4.0	1.7	1-7
Last test SCC (LS)§		4.3	4.5	1.4	2.7-8.7		3.9	4.3	1.2	2.6-7.9
Dry period length (days)		58	58.2	6.0	46-68		58	58.2	6.8	43-79
Breed										
Purebreds	15 (56)					14 (56)				
Crossbreds	12 (44)					11 (44)				
Parity at dry-off										
1	17 (63)					14 (56)				
2+	10 (37)					11 (44)				

*SDCT = selective dry-cow therapy

†BDCT = blanket dry-cow therapy

‡DIM = days-in-milk

§LS = linear score

||Differences between SDCT and BDCT group within the row are not significant, $P > 0.05$

Table 2. Etiology of infection at dry-off and post calving for quarters assigned to a SDCT* or BDCT† program.

Pathogen group	Pathogen recovered	IMI present at DO			IMI present at 1 to 7 DIM		
		SDCT* n = 103	BDCT† n = 92	Total n = 195	SDCT* n = 102	BDCT† n = 91	Total n = 193
Quarters with no growth		65	62	127	59	55	114
Total quarters with IMI		38	30	68	43	36	79
Quarters with mixed IMI		11	5	16	15	18	33
% of all quarters with IMI‡		36.9%	32.6%	34.8%	42.1%	39.6%	40.9%
Gram-positives (no. (%))							
Coagulase-positive staph.	<i>Staphylococcus aureus</i>	2 (4.1)	0 (0.0)	2 (2.4)	1 (1.7)	0 (0.0)	1 (0.9)
Coagulase-negative staph. (CNS)	<i>Staph. chromogenes</i>	8 (16.3)	8 (22.9)	16 (19.0)	4 (7.0)	2 (3.9)	6 (5.5)
	<i>Staph. epidermidis</i>	2 (4.1)	0 (0.0)	2 (2.4)	0 (0.0)	1 (1.9)	1 (0.9)
	<i>Staph. haemolyticus</i>	2 (4.1)	0 (0.0)	2 (2.4)	3 (5.3)	1 (1.9)	4 (3.7)
	<i>Staph. hominis</i>	2 (4.1)	0 (0.0)	2 (2.4)	1 (1.7)	0 (0.0)	1 (0.9)
	<i>Staph. xylosus/saprophyticus</i>	4 (8.2)	5 (14.3)	9 (10.7)	3 (5.3)	2 (3.9)	5 (4.6)
	Other Staph. spp	1 (2.0)	5 (14.3)	6 (7.1)	4 (7.0)	0 (0.0)	4 (3.7)
Environmental Strep. group	<i>Enterococcus casseliflavus</i>	0 (0.0)	1 (2.9)	1 (1.2)	6 (10.5)	6 (11.8)	12 (11.1)
	<i>Enterococcus hirae</i>	0 (0.0)	1 (2.9)	1 (1.2)	-	-	-
	<i>Enterococcus faecalis</i>	-	-	-	1 (1.7)	0 (0.0)	1 (0.9)
	<i>Streptococcus dysgalactiae</i>	1 (2.0)	0 (0.0)	1 (1.2)	-	-	-
	Other Strep. spp	2 (4.1)	2 (5.7)	4 (4.7)	4 (7.0)	3 (5.9)	7 (6.5)
Other gram-positives	<i>Bacillus</i> spp	12 (24.5)	2 (5.7)	14 (16.7)	7 (12.3)	8 (15.7)	15 (13.9)
	<i>Corynebacterium</i> spp	3 (6.1)	3 (8.6)	6 (7.1)	6 (10.5)	6 (11.8)	12 (11.1)
	<i>Aerococcus</i> spp	2 (4.1)	3 (8.6)	5 (6.0)	11 (19.3)	12 (23.5)	23 (21.3)
	<i>Aerococcus viridans</i>	0 (0.0)	2 (5.7)	2 (2.4)	2 (3.4)	6 (11.8)	8 (7.4)
	<i>Trueperella pyogenes</i>	1 (2.0)	0 (0.0)	1 (1.2)	0 (0.0)	1 (1.9)	1 (0.9)
	Gram-positive rods	5 (10.2)	0 (0.0)	5 (6.0)	1 (1.7)	0 (0.0)	1 (0.9)
	Gram-positive cocci	-	-	-	1 (1.7)	0 (0.0)	1 (0.9)
Total gram-positives		47 (96.0)	32 (91.3)	79 (94.0)	55 (96.6)	48 (94.2)	103 (95.4)
Gram-negatives (no. (%))							
Coliforms	<i>Enterobacter</i> spp	0 (0.0)	1 (2.9)	1 (1.2)	-	-	-
	<i>Citrobacter</i> spp	1 (2.0)	0 (0.0)	1 (1.2)	-	-	-
	<i>Serratia marcescens</i>	0 (0.0)	1 (2.9)	1 (1.2)	-	-	-
	<i>Escherichia coli</i>	1 (2.0)	0 (0.0)	1 (1.2)	1 (1.7)	1 (1.9)	2 (1.8)
Other gram-negatives	Gram-negative organisms	0 (0.0)	1 (2.9)	1 (1.2)	0 (0.0)	1 (1.9)	1 (0.9)
Total gram-negatives		2 (4.0)	3 (8.7)	5 (6.0)	1 (1.7)	2 (3.9)	3 (2.8)
Other organisms	Yeast	-	-	-	1 (1.7)	1 (1.9)	2 (1.8)
Total organisms recovered		49 (100)	35 (100)	84 (100)	57 (100)	51 (100)	108 (100)

*SDCT = selective dry-cow therapy program

†BDCT = blanket dry-cow therapy program

‡Differences between SDCT and BDCT group within the row are not significant, $P > 0.05$ *Effect of treatment on risk for developing a new intramammary infection during the dry period*

All quarters enrolled were at risk for developing a new IMI during the dry period. However, 29 quarters that had contaminated samples at DO or PC (SDCT: $n = 11$ and BDCT: $n = 18$) could not be assigned a new infection status and were excluded from the analysis. As such, a total of 179 quarters were used for analysis. The overall proportion of eligible quarters that developed a new IMI between DO and calving was 40.8%, with no difference between the treatment groups (SDCT = 40.2%; BDCT = 41.5%) [$OR_{SDCT} = 0.90$ (95% CI: 0.48, 1.70)] ($P = 0.76$, Table 3). Breed was the only significant covariate in the model, with purebreds

having a lower risk of acquiring new IMI than crossbreds [$OR_{purebreds} = 0.46$ (95% CI: 0.24, 0.86)] ($P = 0.01$).

Effect of treatment on risk for developing clinical mastitis between dry-off and 30 days-in-milk

From a total of 208 quarters that were eligible for a clinical mastitis event, only 4 quarters (1 in BDCT group; 3 in SDCT group) experienced a clinical mastitis event between DO and 30 DIM, with no significant difference between the 2 treatment groups (SDCT = 2.8%; BDCT = 1.0%) ($P = 0.35$).

Antibiotic use and partial budget analysis

The quarter-level prevalence of IMI at DO in the SDCT

Table 3. Results of final multivariable logistic regression models evaluating the effect of adopting a culture-guided, quarter-level selective dry-cow therapy program on measures of udder health as compared to a blanket dry-cow therapy program.

Outcome parameter for models	Treatment group	Crude proportion affected	Odds ratio (95% CI)	P value
Risk for intramammary infection at DO†	SDCT‡	36.9%	1.22 (0.66, 2.29)	0.51
	BDCT§	32.6%	Referent	
Risk for intramammary infection at calving*	SDCT‡	42.2%	1.12 (0.61, 2.05)	0.71
	BDCT§	39.6%	Referent	
Risk for cure between DO† and calving	SDCT‡	82.3%	0.60 (0.11, 3.06)	0.53
	BDCT§	88.0%	Referent	
Risk for new intramammary infection between DO† and calving*	SDCT‡	40.2%	0.91 (0.48, 1.70)	0.76
	BDCT§	41.5%	Referent	

*Model adjusted for breed

†DO = dry-off

‡SDCT = selective dry-cow therapy program

§BDCT = blanket dry-cow therapy program

||Differences between SDCT and BDCT group are not significant, $P > 0.05$

group, according to the rapid culture system, was 52% (56/108). Therefore, 48% (52/108) of the quarters were classified as uninfected and did not receive a long-acting intramammary Ab at DO. By design, 100% of the quarters received Ab in the BDCT group. A partial budget analysis indicated a \$2.77 benefit per cow assigned to the SDCT (Table 4).

Diagnostic test characteristics

The estimates of Se and Sp of the rapid culture system were 82.4% (95% CI: 73.3, 91.4) and 73.2% (95% CI: 65.5, 80.9), respectively. The NPV and PPV was 88.6% (95% CI: 82.5, 94.7) and 62.2% (95% CI: 52.2, 72.2), respectively. The kappa statistic was calculated as 0.517 (95% CI: 0.44, 0.58), indicating a moderate level of agreement between the rapid culture system and the lab culture (gold standard: IMI/no IMI based on routine laboratory culture).

Discussion

The success of a SDCT program to reduce Ab use while maintaining udder health may depend on several factors including, but not limited to, the ability of the test to accurately identify cows or quarters with IMI requiring treatment at DO, use of an ITS to protect untreated quarters, consideration of whether the program targets cow or quarter-level diagnosis and treatment decisions, and prevalence of IMI at DO within the herd. Of these factors, 1 of the most important factors impacting the success of a SDCT program will be to ensure that infected quarters are identified and appropriately treated. Several earlier SDCT studies have used historical records (e.g. cow SCC and clinical mastitis history), cow-side tests (California mastitis test (CMT), N-acetyl-beta-D-glucosaminidase test), and on-farm culture techniques which vary in their ability to accurately identify cows or quarters as candidates for SDCT.^{4,9,23,33,35,37,49} Studies have reported that Se of the CMT to identify infected cows or infected quarters is 70% and

50%, respectively.^{25,41} Similarly, the Se of using a SCC cut-off of $> 200,000$ cells/mL to identify infected cows or quarters has been reported to be 69.7% and 62.4%, respectively.^{29,49} Studies that used these indirect tests to identify IMI at DO have frequently reported that SDCT was not as effective as BDCT programs. For example, Rajala-Schultz et al and Scherpenzeel et al reported that low SCC cows assigned to the SDCT group had significantly higher average SCC in the subsequent lactation, were 1.7 times more likely to experience clinical mastitis, and 1.6 times more likely to experience subclinical mastitis at 14 DIM compared to low SCC cows assigned to BDCT group, respectively. Another recent study predicted outcomes associated with SDCT programs based on 7 SCC cut-off scenarios.⁴⁵ Although all 7 SDCT programs modeled were predicted to reduce Ab usage, they were also predicted to result in more clinical and subclinical mastitis, and so were biologically and economically inferior to BDCT.⁴⁵ These studies suggest that SDCT programs based on indirect tests that lack sufficient Se may have reduced opportunity for success. Failure to use a teat sealant to protect untreated quarters from new IMI during the dry period may be an additional factor negatively affecting the efficacy of several of the aforementioned SDCT studies.

Direct tests, such as rapid culture systems, can have greater Se than indirect tests, and may therefore offer greater opportunities for SDCT success, especially when coupled with use of an ITS to protect untreated cows.¹¹ A recent multi-reader validation study of the Minnesota Easy™ 4Cast™ plate estimated the Se and Sp to be 92.4% and 51.7%, respectively.³⁸ Our evaluation of the Minnesota Easy™ 4Cast™ plate in the current study with a single reader also showed reasonably high Se (82.4%). These results support the idea that rapid on-farm culture systems may be a good test to identify infected quarters.

The current study found that early lactation udder health was not inferior for quarters assigned to the culture-

Table 4. Partial budget analysis for adopting a culture-guided, quarter-level selective dry-cow therapy program as compared to a blanket dry-cow therapy program.

Item	Cost per item (\$)	Added expenses for SDCT program (\$ per cow)	Savings for SDCT program (\$ per cow)	Net return for SDCT program (\$ per cow)
Disposable gloves	\$6.20/box of 100	\$0.12	-	
Milk sample vials (4 per cow)	\$223.63/box of 600	\$1.49	-	
Alcohol wipes to prep teats	\$4.49/packet of 200	\$0.09	-	
Single use swabs to plate milk	\$17/ box of 1000	\$0.07	-	
Rapid culture system plate	\$3.00/plate	\$3.00	-	
Labor	\$15/hour	\$1.87*	-	
Incubator	\$450/incubator	\$0.09†	-	
Intramammary antibiotic	\$4.75/tube	-	\$9.50‡	
Total	-	\$6.73	\$9.50	\$2.77

*Labor cost assumptions: assume 7.5 min/cow to sample the animal plus complete culture activities prior to dry-off. Labor cost/cow = (7.5/60 minutes/hour) * \$15/hr = \$1.87/cow.

†Incubator cost assumptions: assume a large dairy will dry off 800 cows/year, and that purchase of a \$450 incubator to be paid for over a 5-year period. In addition to assuming the incubator will be used for culture of all cows at dry-off (800 cow-uses/year), we also assumed the incubator would be used for rapid culture of clinical mastitis cases during lactation, with 25% of cows experiencing at least 1 clinical mastitis event (25% * 800 = 200 uses/year). Therefore, total cow-uses/year = 1000 (800 + 200) and total cow-uses over 5 years = 5000 (1000/year * 5). Therefore, cost/cow-use = \$450/5000 = \$0.09/cow-use. Aside: a cheaper egg incubator could be used in a smaller herd.

‡Antibiotic savings assumptions: assume will use 4 tubes/cow for the BDCT group, but only 2 tubes/cow (48% reduction in Ab use) for the SDCT group. At a cost of \$4.75/tube for Spectramast DC, total antibiotic savings = [(4*\$4.75) - (2*\$4.75)] = \$9.50/cow dried off.

guided, quarter-level SDCT program, as compared to BDCT. The crude prevalence of IMI at 1 to 7 DIM (38.5%) was within the range reported in other dry-cow mastitis studies.^{1,12,14} The crude proportion of quarters experiencing a cure in this study (84.7%) was high, and similar to that reported in other studies when quarters received Ab and ITS.^{8,12,22,26} The risk of acquiring a new IMI (40.8%) was higher than in previous studies (4.7% to 14.7%).^{1,2,12,14,35} While the incidence of clinical mastitis between DO and 30 DIM was low (1.9%) and not different between BDCT and SDCT, results should be interpreted cautiously given the very small number (n = 4) of clinical mastitis cases between DO and 30 DIM. A post-hoc power analysis indicated that the sample size in this study (approximately 100 quarters per treatment group) provided in excess of 95% confidence and 80% power to detect a difference in prevalence of IMI at calving and incidence of new IMI of 40% and 60%, to detect a difference in cure rate of 80% and 94%, to detect a difference in incidence of clinical mastitis of 1% and 11% in the BDCT and SDCT groups, respectively. The authors acknowledge that this pilot study had an insufficient sample size to detect smaller numeric differences between treatments. However, given the small numeric differences actually observed, we believe there is merit in this approach to quarter-level, culture-guided SDCT, and recommend a larger study be completed in the future.

One concern that could theoretically compromise the success of a quarter-level SDCT program is the fact that there is interdependence among quarters for acquisition of new IMI during the dry period.^{3,36} This could result in increased risk

for infection in an uninfected, untreated quarter adjacent to an infected quarter. It is possible that this interdependence among quarters could have played a role in earlier studies that reported a higher risk of new IMI in SDCT than BDCT cows,^{2,35,45} given that untreated cows in these studies were left unprotected during the dry period. However, other studies indicate the impact of interdependence among quarters can be reduced when effective prevention strategies are in place (e.g. use of ITS) or when the risk of cross-contamination is low.^{2,3,7,24,26,36,40} Internal teat sealants are made of inert substance that has no Ab property; therefore when used alone, it is essential to minimize the risk of introduction of bacteria by following strict asepsis and partial infusion techniques. In the current study, all dry cow treatments were performed by the study personnel and aseptic procedures were followed. The positive results attained in this pilot study support the hypothesis that ITS can minimize or negate the risk of cross-infection between adjacent quarters during the dry period.

Effect of selective dry cow therapy on antibiotic use and partial budget analysis

In this pilot study, the SDCT was not inferior to BDCT in maintaining udder health and also reduced Ab usage by 48%. Partial budget analysis resulted in a savings of \$2.77 per cow. However, it is important to note that the savings per cow might vary among different herds depending on the time taken to sample a single cow and complete all the culture activities, as well as labor costs. It is important to acknowledge that the magnitude (and direction) of this positive impact

of SDCT will vary among herds due to a variety of factors including accuracy (Se/Sp) of the test used, cost of the test program, cost of the dry cow Ab used, and prevalence of IMI at DO in the herd. However, if herds are interested in adopting a SDCT program, or if the Food and Drug Administration (FDA) eventually mandates that US dairy herds must adopt SDCT programs, as has already been done in some European countries, then the results of this pilot study may be useful to reassure producers that SDCT may be at least economically neutral, with a small positive economic impact observed in this study.

Study strengths and limitations

Our goal was to perform a proof-of-concept pilot study to evaluate the safety and efficacy of adopting a culture-guided SDCT program that targeted diagnosis and treatment of individual quarters at the time of DO. The major strength of this study is that this is the first time a rapid culture system and ITS have been used in combination to make quarter-level diagnosis and treatment decisions. This allowed us to maintain udder health while further reducing Ab use as compared to a previous cow-level SDCT program.¹² Although the study was only conducted in 1 herd, the pathogen profile observed for IMI at DO was similar to that reported for other studies. For example, Pantoja et al and Arruda et al reported that CNS was responsible for 63% and 53.9% of infections at DO, respectively. Similarly, relatively few gram-negative pathogens and very few *Staphylococcus aureus* infections were detected in the current study, which is consistent to previous studies reporting variable rates of subclinical IMI caused by gram-negative pathogens, ranging from <1% to 20.4%.^{1,12,17,29}

An obvious limitation of this pilot study is the relatively small number of quarters (~ 100/group), resulting in insufficient power to detect smaller differences between treatments. However, the results were very positive in that we observed very small numeric differences and no statistically significant differences in udder health parameters between the 2 study groups, while reducing Ab use by 48%. Another limitation in the study is the use of results from a single quarter milk sample as a gold standard to identify IMI. Dohoo et al reported that for all bacterial species except *S. aureus*, the Se was <90% (and in many cases <50%) when using culture results from a single milk sample to detect IMI (as compared to 3 consecutive samples). With the exception of CNS, the Sp exceeded 94% for most species. Based on their results, those authors recommended that when using culture results from a single milk sample (0.01 mL volume plated) to determine IMI status, 1 or more colonies could be considered significant for any organism with the exception of CNS, for which 2 or more colonies were recommended to declare IMI present. Because of the lower Se of using a single colony from a single milk sample to determine the IMI status of a quarter, the true prevalence of IMI may be underreported. However, because the same definition of IMI was applied to both treatment groups, there should be no introduction of

bias, thereby allowing for a fair comparison. Several previously published field studies have used a single milk sample to determine the IMI status.^{1,11,19} A necessary next step will be the completion of larger studies using a non-inferiority design, in multiple herds, and with larger numbers of cows, to investigate if results of this study are repeatable, and to investigate variability in efficacy and economics of adopting this program in different herds.

Conclusions

Use of a culture-guided SDCT program that applied diagnosis and treatment decisions at the quarter level resulted in post-calving udder health that was similar to a BDCT program, while reducing Ab use at DO by 48%. A next step will be to verify that this approach can be successful when applied in multiple commercial herds under different management conditions.

Endnotes

^a3M petrifilm rapid culture system

^bMinnesota Easy™ 4Cast™, University of Minnesota, St. Paul, MN

^cOrbeseal, Zoetis, Florham Park, NJ

^dExcel; Microsoft Corp., Redmond, WA

^eNational Mastitis Council

^fMALDI-TOF Braker Daltonics, Bremen, Germany

^gSpectramast DC, Zoetis, Florham Park, NJ

^hMastitis Research Workers' Conference, Nov. 1, 2011, Chicago, IL, chaired by B. Owens, Louisiana State University Agricultural Center, Hill Farm Research Station, Homer

ⁱSAS (version 9.4), SAS Institute Inc., Cary, NC

Acknowledgements

The research was funded by the College of Veterinary Medicine, University of Minnesota. We thank Zoetis Animal Health, Florham Park, NJ, USA for their in-kind support (donation of antibiotic and internal teat sealant). Finally we also thank the management and staff at the St. Paul campus dairy barn for allowing us to perform the study. The authors declare no conflict of interest.

References

1. Arruda AG, Godden SM, Rapnicki P, Gorden P, Timms L, Aly SS, Lehenbauer TW, Champagne J. Randomized non-inferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: Quarter level outcomes. *J Dairy Sci* 2013; 96:4419-4435.
2. Berry EA, Hillerton JE. The effect of selective dry cow treatment on new intramammary infections. *J Dairy Sci* 2002; 85:112-121.
3. Berry EA, Johnston WT, Hillerton JE. Prophylactic effects of two selective dry cow strategies accounting for interdependence of quarter. *J Dairy Sci* 2003; 86:3912-3919.
4. Bhutto AL, Murray RD, Woldehiwet Z. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. *Res Vet Sci* 2012; 92:13-17.

5. Bradley A. Bovine mastitis: an evolving disease. *Vet J* 2002; 164:116-1128.
6. Bradley AJ, Green MJ. The importance of the nonlactating period in the epidemiology of intramammary infection and strategies for prevention. *Vet Clin North Am Food Anim Pract* 2004; 20:547-568.
7. Bradley AJ, Breen JE, Payne B, Williams P, Green MJ. The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. *J Dairy Sci* 2010; 93:1566-1577.
8. Bradley AJ, Breen JE, Payne B, Green MJ. A comparison of broad-spectrum and narrow-spectrum dry cow therapy used alone and in combination with a teat sealant. *J Dairy Sci* 2011; 94:692-704.
9. Browning JW, Mein GA, Barton M, Nicholls TJ, Brightling P. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. *Aus Vet J* 1990; 67:440-442.
10. Call DR, Davis MA, Sawant AA. Antimicrobial resistance in beef and dairy cattle production. *Anim Health Res Rev* 2008; 9:159-167.
11. Cameron M, Keefe GP, Roy JP, Dohoo IR, Macdonald KA, McKenna SL. Evaluation of a 3M Petrifilm on-farm culture system for the detection of intramammary infection at the end of lactation. *Prev Vet Med* 2013; 111:1-9.
12. Cameron M, McKenna SL, Macdonald KA, Dohoo IR, Roy JP, Keefe GP. Evaluation of selective dry cow treatment following on-farm culture: risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. *J Dairy Sci* 2014; 97:270-284.
13. Cook NB, Pionek D, Sharp P. An assessment of the benefits of Orbeseal when used in combination with dry cow antibiotic therapy in three commercial dairy herds. *Bov Pract* 2005; 39:83-94.
14. Dingwell RT, Leslie KE, Schukken YH, Sargeant JM, Timms LL, Duffield TF, Keefe GP, Kelton DF, Lissemore KD, Conklin J. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. *Prev Vet Med* 2004; 63:75-89.
15. Dohoo IR, Martin SW, Stryhn H. Screening and diagnostic tests. In: McPike S, ed. *Veterinary epidemiologic research*. 2nd ed. Charlottetown, Prince Edward Island: VER Inc., 2009; 97-98.
16. Dohoo IR, Smith J, Andersen S, Kelton DF, Godden SM, Mastitis Research Workers' Conference. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. *J Dairy Sci* 2011; 94:250-261.
17. Erskine RJ, Cullor JC, Schaellibaum M, Yancey B, Zeconi A. Bovine mastitis pathogens and trends in resistance to antibacterial drugs. *Proceedings*. 43rd Annu Meeting NMC. 2004; 400-414.
18. Erskine RJ, Walker RD, Bolin CA, Bartlett PC, White DG. Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. *J Dairy Sci* 2002; 85:1111-1118.
19. Godden SM, Rapnicki P, Stewart S, Fetrow J, Johnson A, Bey R, Farnsworth R. Effectiveness of an internal teat seal in the prevention of new intramammary infections during the dry and early-lactation periods in dairy cows when used with a dry cow intramammary antibiotic. *J Dairy Sci* 2003; 86:3899-3911.
20. Green MJ, Green LE, Medley GF, Schukken YH, Bradley AJ. Influence of dry period bacterial intramammary infection on clinical mastitis in dairy cows. *J Dairy Sci* 2002; 85:2589-2599.
21. Green MJ, Bradley AJ, Medley GF, Browne WJ. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. *J Dairy Sci* 2007; 90:3764-3776.
22. Gundelach Y, Kalscheuer E, Hamann H, Hoedemaker M. Risk factors associated with bacteriological cure, new infection, and incidence of clinical mastitis after dry cow therapy with three different antibiotics. *J Vet Sci* 2011; 12:227-233.
23. Hassan Z, Daniel RC, O'Boyle D, Frost AJ. Effects of dry cow intramammary therapy on quarter infections in the dry period. *Vet Rec* 1999; 145:635-639.
24. Huxley JN, Green MJ, Green LE, Bradley AJ. Evaluation of the efficacy of an internal teat sealer during the dry period. *J Dairy Sci* 2002; 85:551-561.
25. Middleton JR, Hardin D, Steevens B, Randle R, Tyler JW. Use of somatic cell counts and California mastitis test results from individual quarter milk samples to detect subclinical intramammary infection in dairy cattle from a herd with a high bulk tank somatic cell count. *J Am Vet Med Assoc* 2004; 224:419-423.
26. Newton HT, Green MJ, Benchaoui H, Cracknell V, Rowan T, Bradley AJ. Comparison of the efficacy of cloxacillin alone and cloxacillin combined with an internal teat sealant for dry-cow therapy. *Vet Rec* 2008; 162:678-684.
27. NMC (National Mastitis Council). *Laboratory Handbook on Bovine Mastitis*. Rev ed. National Mastitis Council Inc., Madison, WI, 1999.
28. Oliver SP, Murinda SE, Jayarao BM. Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: A comprehensive review. *Foodborne Pathog Dis* 2011; 8:337-355.
29. Pantoja JCF, Hultand C, Ruegg PL. Dynamics of somatic cell counts and intramammary infections across the dry period. *Prev Vet Med* 2009; 90:43-54.
30. Pitkala A, Haveri, M, Pyoral S, Myllys V, Honkanen-Buzalski T. Bovine mastitis in Finland 2001 - prevalence, distribution of bacteria, and antimicrobial resistance. *J Dairy Sci* 2004; 87: 2433-2441.
31. Pol M, Ruegg PL. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. *J Dairy Sci* 2007; 90:249-261.
32. Pol M, Ruegg PL. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. *J Dairy Sci* 2007; 90:262-273.
33. Rajala-Schultz PJ, Torres AH, Degraives FJ. Milk yield and somatic cell count during the following lactation after selective treatment of cows at dry-off. *J Dairy Res* 2011; 78:489-499.
34. Reyher KK, Dufour S, Barkema HW, Des Coteaux L, DeVries TJ, Dohoo IR, Keefe GP, Roy JP, Scholl DT. The National Cohort of Dairy Farms - A data collection platform for mastitis research in Canada. *J Dairy Sci* 2011; 94:1616-1626.
35. Rindsig RB, Rodewald RG, Smith AR, Spahr SL. Complete versus selective dry cow therapy for mastitis control. *J Dairy Sci* 1978; 61:1483-1497.
36. Robert A, Bareille N, Roussel P, Poutrel B, Heuchel V, Seegers H. Interdependence of udder quarters for new intramammary infection during the dry period in cows submitted to selective antibiotic therapy. *J Dairy Res* 2006; 73:345-352.
37. Robinson TC, Jackson ER, Marr A. Mastitis incidence in quarters with different infections status at drying off and calving in two treatment groups. *Br Vet J* 1988; 144:166-173.
38. Royster E. Information and updates from the laboratory for udder health. *Proceedings*. MN Dairy Health Conference. May 19-20, 2016. Bloomington, MN.
39. Ruegg PL. New perspectives in udder health management. *Vet Clin North Am Food Anim Pract* 2012; 28:149-163.
40. Sanford CJ, Keefe GP, Dohoo IR, Leslie KE, Dingwell RT, DesCoteaux L, Barkema HW. Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J Am Vet Med Assoc* 2006; 228:1565-1573.
41. Sargent JM, Leslie KE, Shirley JE, Pulkrabek BJ, Lim GH. Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation. *J Dairy Sci* 2001; 84:2018-2024.
42. Savage E, Chothe S, Lintner V, Pierre T, Matthews T, Kariyawasam S, Miller D, Tiwari D, Jayarao B. Evaluation of three bacterial identification systems for species identification of bacteria isolated from bovine mastitis and bulk tank milk samples. *Foodborne Pathog Dis* 2017; In press.
43. Scheepers AJ, Lam TJGM, Schukken YH, Wilmink JBM, Hanekamp WJA. Estimation of various components for somatic cell counts to determine thresholds for uninfected quarters. *J Dairy Sci* 1996; 80:1833-1840.
44. Scherpenzeel CGM., den Uijl IEM, van Schaik G, Olde Riekerink RGM, Keurentjes JM, Lam TJGM. Evaluation of the use of dry cow antibiotics in low somatic cell count cows. *J Dairy Sci* 2014; 97:3606-3614.
45. Scherpenzeel CGM, den Uijl IEM, van Schaik G, Olde Riekerink RGM, Hogeveen H, TJGM. Effect of different scenarios for selective dry-cow therapy on udder health, antimicrobial usage and economics. *J Dairy Sci* 2016; 99:3753-3764.
46. Schukken YH, Vanvliet J, Vandegheer D, Grommers FJ. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herd. *J Dairy Sci* 1993; 76:2925-2930.
47. Smith KL, Todhunter DA, Schoenberger PS. Environmental pathogens and intramammary infection during the dry period. *J Dairy Sci* 1985; 68:402-417.
48. Tenhagen BA, Koster G, Wallmann J, Heuwieser W. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *J Dairy Sci* 2006; 89: 2542-2551.
49. Torres AH, Rajala-Schultz PJ, Degraives FJ, Hoblet KH. Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off. *J Dairy Res* 2008; 75:240-247.