An observational cohort study on persistency of internal teat sealant residues in milk after calving in dairy cows

Fidèle Kabera,*† Simon Dufour,*† Greg Keefe,†‡ and Jean-Philippe Roy†§¹

*Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada J2S 2M2

†Canadian Bovine Mastitis and Milk Quality Research Network, Saint-Hyacinthe, Québec, Canada J2S 2M2

‡Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada C1A 4P3

§Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada J2S 2M2

ABSTRACT

Our objectives were to evaluate the prevalence of quarters with an observable internal teat sealant (ITS) plug at first milking following calving and investigate persistency of ITS residues in milk after calving. An observational cohort study was carried out on 557 quarters of 156 cows treated with ITS in 6 farms in Quebec, Canada. The presence of an ITS plug at first milking and ITS residues in milk at each milking were observed by producers. The effects of various factors on the odds of observing an ITS plug and persistency of ITS residues in milk were studied using generalized logistic mixed and generalized negative binomial mixed models, respectively. Milk samples were taken on the day before dry-off and on 2 occasions after calving for bacterial identification to detect intramammary infection (IMI) using bacteriological culture followed by MALDI-TOF identification. The association between the absence of an ITS plug and the presence of new IMI was assessed using a mixed logistic regression model. Internal teat sealant plugs after calving were more often observed in rear quarters and in quarters receiving ITS alone at drying-off versus antimicrobial and ITS. We observed an average (standard deviation) persistency of 4.0 d (2.3 d). When an ITS plug was still present at first milking (83% of quarters), the elimination of ITS residues in milk after calving was significantly longer (4.5 d, on average) compared with 1.2 d when an ITS plug was absent. In cows with an ITS plug at calving, we observed a higher number of days of excretion in older cows. When a plug could not be observed, rear quarters, older cows, and cows with a long dry period duration excreted ITS residues for a significantly longer

period. The lack of a significant association between the absence of a plug and the odds of new IMI at calving suggests that despite the loss of the plug, cows were still protected against new IMI. Although we were able to highlight some statistically significant risk factors explaining persistency of ITS residues following calving, observed differences were often relatively small and, perhaps, not clinically relevant. In conclusion, an ITS plug was present until first milking after calving for 83% quarters, quarters without an ITS plug at first milking appeared to have been protected from new IMI, and ITS residues could be observed in milk up to 12 d in milk.

Key words: internal teat sealant, residue, intramammary infection, calving, dry period

INTRODUCTION

Dairy cows are at high risk of developing new IMI (NIMI) during the dry period, which often remain undetected until calving or even a long time after calving in some cases (Smith et al., 1985; Bradley, 2002). These dry-period-acquired NIMI combined with IMI that persist from the previous lactation are important determinants of the IMI prevalence in the subsequent lactation (Green et al., 2002). Factors that influence the susceptibility to NIMI at the beginning of the dry period include the functional transition associated with mammary involution, the delay in the complete formation of keratin plug in the teat canal, and the cessation of teat sanitization (Smith et al., 1985; Dingwell et al., 2003; Halasa et al., 2009). In fact, a previous study in New Zealand observed that 50% of teats did not develop a keratin plug during the first 10 d of the dry period (Williamson et al., 1995). Furthermore, other studies reported that 23% of teats were still open up to 6 wk after drying off and, for 3 to 5% of teats, a keratin plug was never observed (Williamson et al., 1995; Dingwell et al., 2004).

Received October 12, 2017.

Accepted February 25, 2018.

¹Corresponding author: jean-philippe.roy@umontreal.ca

To prevent NIMI during the dry period, the application of an internal teat sealant (**ITS**) alone or combined with the administration of antimicrobial is now widely used. An ITS forms a physical barrier to the entry of bacteria responsible for mastitis and thus reduces the risk of NIMI occurring during the dry period (Berry and Hillerton, 2002a; Godden et al., 2003; Sanford et al., 2006). For example, Orbeseal (Zoetis Canada, Kirkland, Quebec, Canada) is an ITS consisting of bismuth subnitrate formulated into an inert viscous malleable paste. It is a sterile and non-antimicrobial intramammary infusion. Use of ITS can complement or provide an alternative to antimicrobial dry cow therapy to protect quarters during the dry period (Woolford et al., 1998; Berry and Hillerton, 2002b; Huxley et al., 2002). Such strategy, which does not involve antimicrobials, is of considerable importance because of public health concerns on antimicrobial resistance and antimicrobial residues in milk. The ITS is not absorbed systemically from the mammary gland and can persist in the teat for at least 100 d during the dry period (Woolford et al., 1998). It remains in the teat cistern over the dry period until it is physically removed manually at first milking, or by suckling by the calf.

Currently, few studies have evaluated the proportion of quarters at first milking after calving that still have an ITS plug. The proportion of quarters truly protected through the entire dry-off period is, therefore, not well described. Furthermore, it is not clear whether quarters, having lost the sealant plug before first milking, were still substantially protected from NIMI during the dry period. Finally, the risk factors that may influence the persistence of sealant plug until the first milking are not well described.

Moreover, few data are currently available on the persistency of ITS residues in milk after calving and on factors affecting ITS excretion following calving have not been reported. Some authors reported presence of sealant residues in milk up to 3 wk after calving (Berry and Hillerton (2002a). Bhutto et al. (2011) reported that most of the product was eliminated at the first milking, but that some residues may be observed over the subsequent milkings. However, these authors did not investigate the average duration of residue excretion.

Consequently, the primary objectives of the current study were to (1) quantify prevalence of quarters with an observable sealant plug at first milking following calving, (2) investigate persistency of ITS residues in milk after calving, and (3) identify risk factors that could affect presence of an ITS plug at calving and number of days of ITS excretion after calving. A secondary objective of the study was to investigate whether

quarters without an observable sealant plug at the first milking after calving were equally protected from NIMI acquisition during the dry period compared with those with an observable sealant plug.

MATERIALS AND METHODS

Participants

The current study was an observational cohort study performed on 557 quarters from 156 cows treated with ITS in 6 dairy farms in Quebec (Canada) between October 2015 and July 2016. This cohort of 6 farms was a convenience sample from a larger randomized controlled trial (**RCT**) on quarter-based selective dry cow therapy conducted on 9 farms. From that larger sample, only farms where the milking staff agreed to record the presence of residues in milk following calving were selected. For the RCT, herd inclusion criteria were (1) a bulk tank SCC mean <250,000 cells/mL over the last year, (2) a targeted dry period of 35 to 75 d, (3) participation in a DHI program, and (4) willingness to commit to the project protocol. In these herds, all pregnant dairy cows ready for drying-off, having at least 3 functional quarters, and not treated with antimicrobials during the 14 d before dry-off were enrolled. Cows that failed to meet the inclusion criteria were treated as per routine farm procedures for dry-off.

In the RCT, a total of 574 cows were recruited and allocated, using a random number generator, to 4 groups: (1) intramammary (**IMM**) infusion of dry cow antimicrobial therapy alone; (2) IMM infusion of dry cow antimicrobial therapy and ITS; (3) on-farm culture using a Petrifilm Aerobic Count Plate (3M, London, Ontario, Canada) with positive quarters (defined as ≥ 5 cfu/mL on the Petrifilm Aerobic Count Plate) treated with IMM infusion of dry cow antimicrobial therapy alone and negative quarters treated with ITS alone; and (4) on-farm culture using Petrifilm with positive quarters treated with IMM infusion of dry cow antimicrobial therapy and ITS and negative quarters treated with ITS alone. Farm staff were blinded to treatment allocation, and therefore, they could not choose a group for a cow or keep her out of the study.

For the current cohort study, conducted on 6 of these farms, only quarters dry treated with ITS alone, or with an antimicrobial and ITS were selected. These quarters received, based on group allocation and dry-off IMI status, 4 g of an ITS containing 65% wt/wt of bismuth subnitrate (Orbeseal) with or without an IMM infusion of dry cow antimicrobial (200,000 IU of Penicillin G Procaine and 400 mg of Novobiocin; Novodoxy Plus, Zoetis Canada, Kirkland, Quebec, Canada). All

treatments were applied by farm personnel immediately after the last milking before dry-off, following the procedures recommended on the Canadian Bovine Mastitis and Milk Quality Research Network's factsheet on administration technique for IMM treatment in dairy cows (CBMQRN, 2010). Treatments were recorded to verify compliance with the study protocol.

Data Collection

Before the start of the study, each participant was trained in aseptic IMM infusion techniques when using ITS, the stripping out of the ITS after calving, and the observation of ITS residues before milking. In addition, a practical illustrated sheet on administration technique for IMM treatment in dairy cattle was provided, explained, and left on farms. The research team visited participating farms every other week throughout the course of the study for enrollment of cows at dry-off and for monitoring the progress and respect of the study protocol. In each farm, 1 (the owner, in general) or 2 employees (the owner and 1 employee) were allocated to the project and collected all the observations.

The presence of an observable ITS plug at first milking following calving and persistency of ITS residues in milk after calving were monitored visually by dairy producers.

An ITS plug was considered effective if the pressure on the teat required to remove the plug was greater than required for normal milk removal, and significant ITS was observed following stripping. Residues were observed directly on the floor or using a filter-cup at each milking, depending on milkers' preferences. The monitoring of residues in milk was pursued until observation of at least 4 successive milkings without residues. The last day that residues were observed in each quarter was used to calculate the number of days with residues for statistical analyses.

Putative Risk Factors. Data on parity, milk production at drying off, and milk production in the early lactation were extracted from farm milking system software (1 herd) and from monthly DHI data (5 herds) and investigated as potential predictors of ITS excretion. For milk production at drying off, the measurement collected on last DHI test before drying-off was used. For milk production in the early lactation, we hypothesized that milk production in the first few DIM may affect the presence of an ITS plug or days of ITS excretion or both. For many cows, however, the first milk production measurement was that of DHI control and this measurement could be collected sometimes very early in the lactation, but sometimes up to 40 to 45 DIM (depending on concordance between calving

date and next scheduled DHI control). Therefore, to get a more stable, homogeneous, and reliable measure, instead of using only the first milk production measurement reported, we used the mean milk production of the first 15 wk in milk for the farm from which we could get weekly milk production data, and the mean milk production of the first 3 DHI tests following calving for the other farms. Our hypothesis was that high-producing cows in the first 15 wk in milk were possibly also high-producing cows around calving. Parity was categorized based on its distribution into 2nd parity, 3rd parity, and ≥ 4 th parity. Information regarding the residue visualization method used (filter-cup vs. floor) was provided by the participating producers.

Milk Samples for Bacteriological Analyses. Using aseptic sample technique (National Mastitis Council, 2017a), single quarter milk samples of all enrolled cows were collected on the day before dry-off (S_1), and after calving at 3 to 4 DIM (S_2), and 5 to 18 DIM (S_3) to detect IMI using bacteriological culture followed by MALDI-TOF MS identification. Sample S_2 was collected by the producer or farm personnel, whereas S_1 and S_3 samples were collected by the research team. Milk samples were frozen at -20°C before a monthly shipment to the Maritime Quality Milk research laboratory at the University of Prince Edward Island where culture and bacterial identification were conducted. Laboratory personnel were blinded to treatment allocation when conducting bacteriological analyses.

Laboratory Analyses

Milk Bacteriological Culture. Milk samples were thawed and cultured in the Maritime Quality Milk research laboratory using standardized methods outlined in the Laboratory Handbook on Bovine Mastitis (National Mastitis Council, 2017b). Briefly, disposable plastic loops were used to streak 0.01 mL of milk on bi-plates containing half Columbia agar + 5% sheep blood and half MacConkey agar. Plates were incubated at 35°C and examined for bacterial growth after 24 and 48 h. Colonies were tentatively identified as staphylococci, streptococci, coliforms, or other pathogens based on colony growth characteristics, morphology, pattern of hemolysis, catalase reaction, and Gram stain. For each positive sample, the number of colony-forming units per 0.01 mL of milk was enumerated up to a maximum of 10 colonies. The identification of all microorganisms recovered on the plate was attempted, regardless of the number of colonies. Samples with 3 or more phenotypically different colony types were classified as contaminated. However, if a contaminated sample had one or more hemolytic colonies (suspected

to be *Staphylococcus aureus*), the hemolytic colonies were enumerated and analyzed further. Yeast and *Prototheca* spp. were recorded based on Gram stain results. Colonies of bacteria were subcultured onto blood agar plates to obtain pure culture for further classification using MALDI-TOF MS.

MALDI-TOF MS. Final isolates identification was carried out using the direct transfer method (MALDI Biotyper 3.1 User Manual, Bruker Daltonics Inc., Billerica, MA). Briefly, a single-use, 15-cm sterile wooden applicator stick was used to lift material from a well-isolated bacterial colony followed by smearing a thin film of colony material onto a ground steel MSP 96-spot target (Bruker Daltonics Inc.). The spots were allowed to air dry at room temperature. Subsequently, the spots were overlaid with 1.0 μ L of a saturated solution of α -cyano-4-hydroxycinnamic acid matrix in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid (Sigma-Aldrich Canada Inc., Oakville, ON, Canada) using single-use pipette tips and air-dried at room temperature.

All targets were calibrated using Bacterial Test Standard (Bruker Daltonics Inc.) and included *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 control classification samples in duplicate. To confirm that target cleaning was effective and no residual bacterial material from a previous run remained, one spot on each target contained only matrix with no bacterial sample. All bacterial spectral captures and classifications were carried out using Bruker Daltonics Research Use Only microbial classification platform that included a Microflex LT mass spectrometer, flexControl software (version 3.4), MALDI Biotyper Real Time Classification, Offline Classification (version 3.1) with a 5,627 Main Spectrum reference database library (MBT-BDAL-5627) and a custom CNS library developed by Cameron et al. (2017). All spectra reviews were carried out using flexAnalysis software (version 3.4, Bruker Daltonics Inc.). The MALDI Biotyper RTC was carried out according to the MALDI Biotyper 3.1 User Manual. FlexControl settings were medium mass range (1,960–21,200 m/z), detector gain set to $8.6 \times (3,227 \text{ V})$, sample and digitizer settings at 0.50 GS/s, and ion source 1 and 2 values of 20.12 and 18.25 kV, respectively. Each sample spectrum was summed from a maximum of 240 shots accumulated from a minimum of 6 raster points using a spiral small laser movement pattern.

After acquisition of a sample's mass spectrum, the Biotyper software compares the spectrum to the reference spectra contained in the database. The software then displays matching identifications and computes a score ranging from 0.0 to 3.0, indicating the degree of

similarity between the sample's spectrum and the reference spectrum. Identification scores were interpreted as per manufacturer's recommendations as follows: a score of 2.0 to 3.0 was deemed as acceptable for species-level identification, a score of 1.7 to <2.0 indicated confident identification to the genus level, and a score of <1.7 was considered a nonreliable identification.

Definition of NIMI. To determine whether a NIMI by a specific pathogen was acquired during the dry period, the pre-dry sample (S_1) and first sample after calving (S_2) were used. If the first sample after calving (S_2) was missing or contaminated, then the second sample (S_3) was used. The information on NIMI was, therefore, missing, if sample at drying off (S_1) was contaminated or if both samples after calving (S_2 and S_3) were missing or contaminated. Presence of ≥ 1 cfu/0.01 mL of milk was considered sufficient to qualify a quarter as having an IMI.

If an IMI by a specific pathogen-species was present at calving and if that same pathogen species was not found in the drying off sample, then the quarter was considered as having experienced a NIMI. In our study, groups of bacteria that can be reported by MALDI-TOF analyses, but are not relevant for bovine mastitis were not considered when defining NIMI. In the current study, these were *Corynebacterium amycolatum*, other gram-positive, *Curtobacterium flaccumfaciens*, *Brachybacterium* species, *Brevibacillus parabrevis*, *Kocuria* species, *Weissella confusa*, *Bacillus pumilus*, *Micrococcus luteus*, and *Neisseria flavescens*. Because ITS are used mainly to prevent NIMI by environmental bacteria, we also investigated specifically NIMI by these type of pathogens, by creating a second NIMI definition (**NIMI_{env}**), which further excluded NIMI by contagious mastitis pathogens (*Corynebacterium bovis* and other nonspeciased *Corynebacterium* species, and *Staphylococcus aureus*). Note that *Streptococcus agalactiae*, *Mycoplasma bovis*, and other *Mycoplasma* species were not recovered in the current study.

Statistical Analyses

The primary experimental unit was the quarter. Four outcomes of interest were investigated: (1) presence of an observable ITS plug at first milking after calving; (2) number of days of persistency of ITS residues in milk after calving; (3) development of a NIMI over the dry period; and (4) development of NIMI_{env} during the dry period.

Descriptive statistics were first computed, then the unconditional effect of the different expositions was tested in generalized linear mixed models using SAS Proc GLIMMIX procedure (version 9.4, SAS Institute

Inc., Cary, NC). As observations were clustered within cows (4 quarters per cow) and within herds, random intercepts for cow and herd were included in all statistical models. Models used for the different outcomes are described in the following sections. Assumption of linearity between quantitative predictors and outcomes was evaluated using polynomial terms.

Conditional associations were then computed by introducing the putative confounders in the model; conditional and unconditional associations were then compared and confounders were retained only if measure of association (i.e., odds ratio or incidence ratio) differed by more than 10%, as suggested by Mickey and Greenland (1989). For all models, the significance threshold was set at 0.05. A posteriori adjustments for multiple comparisons were used for predictors >2 categories using the Tukey-Kramer method. Only subjects with complete information on all variables in the final model were included in the analyses (i.e., complete case analysis).

Risk Factors Affecting Odds of an Observable ITS Plug at the First Milking After Calving. A generalized mixed model with a logit link was used to investigate the effect of the different predictors on odds of observing an ITS plug at first milking following calving. Predictors evaluated were parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), duration of dry period, and quarter position (front vs. rear). Parity was investigated as a putative confounder for the association between milk production at drying off and odds of observing an ITS plug at calving and for the association between early lactation milk production and odds of a plug. Also, milk production at drying off was investigated as a potential confounder for the association between the duration of the dry period and odds of observing an ITS plug at calving.

Risk Factors for Number of Days of Persistency of ITS Residues in Milk After Calving. For the number of days of persistency of ITS residues in milk after calving, 2 strikingly different distributions were observed for quarters for which a plug was or was not observed at calving. Therefore, for this outcome, separate analyses were carried out for quarters with an observable ITS plug and quarters without a plug. For these analyses, generalized mixed models using a log link were used to investigate the effect of the different predictors on number of days of ITS residues following calving. For quarters with an observable ITS plug at calving, we investigated whether a Poisson or negative binomial regression model would best fit the data using a *t*-test investigating whether the dispersion parameter

was different from 0. The Poisson regression model offered the best fit and was retained for these analyses. For quarters without an observable ITS plug, we first investigated whether a Poisson or negative binomial would best fit the data, and then, based on the observed distribution, we evaluated whether a zero-inflated or conventional model would provide the best fit using the Vuong test (Vuong, 1989). The conventional negative binomial offered the best fit and was retained for these analyses.

Parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), duration of dry period, quarter position (front vs. rear), and visualization method used by the participating producer were evaluated as potential predictors of number of days of excretion. Again, parity was investigated as putative confounder for the association between milk production at drying off and persistence of ITS residues in milk after calving and for the association between early lactation milk production and persistence of ITS residues in milk after calving. Also, milk production at drying off was investigated as potential confounder for the association between duration of the dry period and persistence of ITS residues in milk after calving.

Effect of ITS Plug Retention on Odds of NIMI Over the Dry Period. Two separate generalized mixed models with a logit link were used for modeling the effect of the presence or not of an observable ITS plug at the first milking on odds of NIMI and on odds of NIMI_{env}. The only exposure considered in this model was presence or not of the ITS plug at calving. The following variables were investigated as potential confounder of these relationships: parity, milk production at drying off, milk production in the early lactation, treatment received at drying off (antimicrobial and ITS vs. ITS alone), quarter position (front vs. rear), and dry period duration.

Power Calculation. Because the sample size was already predetermined by the number of available quarters treated with teat sealant from the RCT, we instead evaluated the minimum differences that could be observed given the actual sample size, using an α of 0.05, and with a power of 0.90. For these calculations, we used a power of 0.90 (vs. 0.80) because clustering of quarters by cow and by herd was not accounted for. Thus, true power was probably less than 0.90. For instance, considering a standard deviation for mean excretion of 3 d, we would have been able to detect differences ≥ 0.83 and ≥ 0.94 d between levels of a dichotomic predictor present in 50 or 75% of quarters, respectively. When evaluating the effect of a dichotomic predictor on odds of having an observable ITS plug at calving, assuming

that 85% of quarters will have an observable plug, and that the predictor would be present in either 50% or 75% of quarters, we computed that we could detect, with a power $\geq 90\%$, difference in odds corresponding to an odds ratio of ≥ 2.2 (for the predictor with a 50% distribution) and of ≥ 2.7 (for the predictor with a 75% distribution).

When evaluating the effect of a dichotomic predictor (absence of the plug) on the odds of acquiring a NIMI during the dry period, assuming that 10% of quarters will have a NIMI, and that the predictor would be present in 15% of quarters, we computed that we could detect, with a power $\geq 90\%$, difference in odds corresponding to an odds ratio of ≥ 2.9 .

RESULTS

Descriptive Statistics

Participants. The 6 participating dairy farms with primarily Holstein cows are described in Table 1. In total, 377 cows from the 6 participating farms calved during the cohort study. Two hundred fifty-six of those 377 cows received ITS at least in one quarter. One hundred (100/256) cows were excluded from the study for different reasons: missing data on ITS residues ($n = 73$), cows died before or at calving ($n = 9$), cows aborted ($n = 4$), cows were culled just after calving ($n = 4$), or dry period was <35 or >75 d ($n = 10$). One hundred fifty-six cows (557 quarters) were monitored during this cohort study with an average of 26 (range 11–67) monitored cows per farm. Sixty-seven quarters could not be included in the study because 60 quarters did not receive ITS at drying off (dry cow antimicrobial therapy alone), and 7 quarters were not functional at drying off.

Predictors. Internal teat sealant residues were observed using a filter-cup for 110/557 (20%) of quarters and forestripped milk was observed directly on the floor for 447 quarters (80%). Two hundred eighty-four front quarters (51%) and 273 rear quarters (49%) were observed during this study. Three hundred twenty-one

quarters (57.6%) received a combination of antimicrobial and ITS, whereas 236 quarters (42.4%) received ITS alone at drying off. Two hundred fourteen quarters (38.4%) were from 2nd parity cows, 140 quarters (25.1%) from 3rd parity cows, and 203 quarters (36.5%) from cows with parity ≥ 4 . Mean (SD) daily milk production at drying off was 24.7 kg/d (7.3 kg) with minimum and maximum of 8.6 and 47.4 kg/d, respectively. Mean (SD) daily milk production after calving was 45.2 kg/d (6.6 kg) with a minimum and maximum of 27.1 and 62.1 kg/d, respectively. The mean (SD) dry period duration was 51.6 d (10.3 d) with a minimum and maximum of 35 and 73 d, respectively.

Internal Teat Sealant. An ITS plug was still present at the first milking after calving for 441/531 (83%) of quarters (range by herd: 45–100%), absent in 90 quarters (17%), and 26 observations were missing [3 cows (6 quarters) from farm 4 and 5 cows (20 quarters) from farm 1]. The duration of ITS residues varied between 0 and 12 d, with a mean (SD) of 4.0 d (2.3 d; Figure 1). When considering separately quarters for which a plug could be visualized after calving and those for which the plug was already gone, 2 strikingly different distributions were observed (Figure 1). For quarters for which an ITS plug was still present at the first milking after calving, persistency of ITS residues varied between 1 and 12 d with a mean (SD) of 4.5 d (1.9 d) and appeared to follow an almost normal distribution, or a Poisson or binomial negative distribution. For quarters without an ITS plug at the first milking after calving, persistency of ITS residues varied between 0 and 8 d with a mean (SD) of 1.2 d (1.7 d) and followed a typical count data distribution (i.e., Poisson or binomial negative with lower mean).

NIMI at Calving. New intramammary infections were observed in 87/530 of quarters (16.4%) and absent in 443 quarters (83.6%), according to the first definition excluding only bacteria not relevant for mastitis. There were 27 missing observations because (1) samples at drying off were contaminated ($n = 21$), or (2) both samples after calving were missing or contaminated ($n = 6$). When considering environmental mastitis patho-

Table 1. Description of 6 dairy farms selected in Québec (Canada) for the study on persistency of internal teat sealant (ITS) residues in milk after calving

Farm	No. of lactating cows	Housing of dairy cows	305-d mean milk production (kg)	No. of cows with missing data	% of quarters with ITS plug at calving
1	80	Tiestall	9,820	7	88
2	200	Freestall	9,687	21	95
3	100	Freestall	9,050	7	45
4	80	Freestall	9,679	7	81
5	140	Freestall	10,149	24	59
6	50	Tiestall	9,614	7	100

gens only, NIMI_{env} were present in 60 of 530 quarters (11.3%) and absent in 470 quarters (88.7%). Table 2 presents the information on pathogen species isolated in cases of NIMI or NIMI_{env}.

Risk Factors Affecting Odds of an Observable ITS Plug at the First Milking After Calving. Associations between predictors and odds of having an observable ITS plug at the first milking after calving are presented in Table 3. Only type of treatment administered at drying-off and quarter position were significantly associated with odds of having an observable ITS plug at calving. Quarters that received ITS alone had 2.6 times greater odds (95% CI: 1.1, 6.1) of having an ITS plug after calving than those that received a combination of antimicrobial and ITS. Rear quarters had 2.1 times greater odds (95% CI: 1.1, 3.9) of having a plug after calving than front quarters.

Risk Factors for Number of Days of Persistency of ITS Residues in Milk After Calving

Quarters with an ITS Plug Present at Calving. Associations between predictors and number of days of persistency of ITS residues in milk after calving in

quarters with an observable ITS plug at calving are presented in Table 4. When an ITS plug was still present at the first milking after calving, Poisson regression with random effects offered the best fit and was used to evaluate the effects of different predictors on the number of days of persistence of ITS residues in milk. Only cow's parity influenced significantly the number of days of excretion of ITS residues in milk after calving. After adjusting for multiple comparisons, quarters of cows ≥ 4 th parity had 1.3 times more days with residues (95% CI: 1.0, 1.5) than quarters from 3rd parity cows. Quarters of cows ≥ 4 th parity also had 1.2 times more days with residues (95% CI: 1.0, 1.4) than quarters from 2nd parity cows. Number of days of residues were not statistically different between quarters from 3rd and 2nd parity cows. Predicted number of days of residues were 3.9, 3.7, and 4.9 d for 2nd, 3rd, and ≥ 4 th parity cows, respectively.

Quarters Without an ITS Plug at Calving. Associations between predictors and number of days of persistency of ITS residues in milk after calving in quarters without an observable ITS plug at calving are presented in Table 5. When an ITS plug was not present at the first milking after calving, negative binomial

Figure 1. Distribution of number of days with internal teat sealant (ITS) residues in milk after calving as a function of presence or not of an observable ITS plug at first milking. Color version available online.

regression with random effects offered the best fit and was used to evaluate the effect of different predictors on the number of days of persistence of ITS residues in milk. Parity, duration of the dry period, and quarter position all significantly affected number of days of ITS residues after calving in quarter without an observable ITS plug at calving.

After adjusting for multiple comparisons, quarters of cows ≥ 4 th parity had 7.9 times more days with residues (95% CI:1.4, 44.0) than quarters from 3rd parity cows. Quarters of cows ≥ 4 th parity also had 6.1 times more days with residues (95% CI: 1.3, 27.7) than quarters from 2nd parity cows. We did not observe a significant difference between quarters from 2nd and 3rd parity cows. Predicted number of days of residues were 0.4, 0.3, and 2.6 d for 2nd, 3rd, and ≥ 4 th parity cows, respectively.

The number of days of persistence of ITS residues in milk after calving were multiplied by a factor of 1.7 (95% CI: 1.1, 2.5) for every 1-wk increase of dry period length. For instance, number of days of residues were 0.1, 0.2, and 0.6 for quarters with a dry period of 6, 8, and 10 wk, respectively.

Finally, rear quarters had 1.6 times more days with residues (95% CI: 1.1, 2.4) than front quarters. Pre-

dicted number of days of residues were 0.5, and 0.7 d for front and rear quarters, respectively.

Effect of ITS Plug Retention on Odds of NIMI Over the Dry Period

Associations between presence of an ITS plug at calving and odds of acquiring a NIMI or a NIMI_{env} are presented in Table 6. Treatment at drying-off (anti-microbials and ITS vs. ITS alone) was retained as a significant confounder of the association between presence of a plug at calving and NIMI and NIMI_{env}. After controlling for this confounder, the fact that ITS plug was present or not at calving was not significantly associated with odds of NIMI at calving ($P = 0.33$, odds = 1.4, 95% CI: 0.7, 3.0) nor with odds of NIMI_{env} ($P = 0.54$, odds = 1.3, 95% CI: 0.6, 3.1). When all relevant mastitis pathogens were considered, NIMI rates were 16.2, and 16.7% of quarters when plug was present or absent, respectively. When environmental mastitis pathogens only were considered, NIMI_{env} rates were 11.4, and 10% of quarters when a plug was present or absent, respectively.

DISCUSSION

Participants

A total of 156 cows from 6 participating farms were monitored during this cohort study. Seventy-three cows were not included in the study because producers did not observe and complete the information on the presence of ITS plug and ITS residues in milk. On some farms, when the employee allocated to the project was away, data collection was suspended. Moreover, a significant proportion (45.2%) of these missing data (33 of 73 cows) was registered between April and July 2016 when farmers were busy with field work and compliance with the research protocol was difficult. If the outcomes or exposures studied are strongly influenced by season, then it is possible that our results may apply well to cows calving in the August–March period, but perhaps not as much to cows calving during April–July where most of the missing data were observed. Moreover, because different observers were used to monitor presence of an effective plug and days of residues, some variation in these measures could be expected between observers. Accuracy of these measures related to individual observer was not evaluated in our study. We could expect, however, that the farm random effect would have captured some of these variations (e.g., for farms with just one observer; $n = 4$). Moreover, the fact that method of residue visualization (i.e., filter-cup versus the floor) was not significantly associated with days of residues

Table 2. Pathogens isolated in 87 quarters with a new IMI in a cohort of 557 quarters from 156 cows from 6 commercial dairies in Quebec, Canada¹

Microorganism	Number of isolates
<i>Aerococcus</i> species	13
<i>Aerococcus viridans</i>	1
<i>Bacillus</i> species	1
<i>Corynebacterium</i> species	19
<i>Enterococcus faecalis</i>	2
Other CNS	8
<i>Pseudomonas</i> species	1
<i>Staphylococcus aureus</i>	10
<i>Staphylococcus chromogenes</i>	2
<i>Staphylococcus epidermidis</i>	1
<i>Staphylococcus equorum</i>	5
<i>Staphylococcus haemolyticus</i>	3
<i>Staphylococcus hominis</i>	2
<i>Staphylococcus sciuri</i>	5
<i>Staphylococcus simulans</i>	2
<i>Staphylococcus xylosus</i>	10
<i>Streptococcus dysgalactiae</i>	1
<i>Streptococcus</i> species	2
<i>Trueperella pyogenes</i>	1
Yeast	2
Total	91 ²
Contagious mastitis pathogens	29
Environmental mastitis pathogens	62

¹Pathogens were identified by bacteriological culture followed by MALDI-TOF identification and, when MALDI-TOF species identification was not conclusive, by routine bacteriological methods.

²Among the 87 quarters with new IMI, 4 quarters had new IMI by 2 different species.

Table 3. Results from the generalized logistic mixed models used to model effect of various predictors on odds of having an observable internal teat sealant (ITS) plug at calving in a cohort of 557 quarters, from 156 cows from 6 commercial dairies

Parameter	Estimate	SE	Odds ratio	95% CI	P-value ¹
(1) Parity					
Intercept	1.981	0.772			
Parity					0.08
2	Reference	Reference	Reference	Reference	
3	−0.563	0.555	0.57	0.19, 1.7	
≥4	0.836	0.593	2.3	0.72, 7.4	
Herd variance	2.591	2.059			
Cow variance	2.891	0.692			
(2) Milk production pre-dry					
Intercept ²	1.157	0.880			
Milk production pre-dry ³	0.330	0.188	1.4	0.96, 2.0	0.08
Herd variance	2.737	2.183			
Cow variance	2.808	0.676			
(3) Milk production early lactation					
Intercept ²	1.193	1.421			
Milk production early lactation ³	0.118	0.182	1.1	0.79, 1.6	0.52
Herd variance	2.413	1.957			
Cow variance	2.894	0.691			
(4) Treatment at dry-off					
Intercept	2.683	0.815			
Treatment					0.04
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.936	0.444	2.6	1.1, 6.1	
Herd variance	2.939	2.343			
Cow variance	3.119	0.734			
(5) Dry period duration					
Intercept ⁴	0.517	1.223			
Dry period duration ⁵	0.161	0.196	1.2	0.8, 1.7	0.41
Milk production pre-dry ⁶	0.407	0.214			0.06
Herd variance	3.204	2.553			
Cow variance	2.853	0.688			
(6) Quarter position					
Intercept	2.479	0.763			
Quarter position					0.02
Front	Reference	Reference	Reference	Reference	
Rear	0.741	0.318	2.1	1.1, 3.9	
Herd variance	2.776	2.197			
Cow variance	3.039	0.707			

¹Joint P-value for the group of predictors (type III test).²Intercept for a cow producing 10 kg of milk per day.³Effect for a 5 kg/d increase in milk production.⁴Intercept for a cow with a 35-d dry period and producing 10 kg of milk per day pre-dry.⁵Effect for a 7-d increase in dry period duration.⁶Milk production pre-dry was retained as a confounder of the relationship between dry period duration and odds of having an observable ITS plug at calving (change in odds ratio: 18%).

seems to suggest a certain homogeneity between observers. Still, possible variations in measurement between observers is one drawback from our study.

Persistency of Internal Teat Sealant Plug and NIMI over the Dry Period

The prevalence of quarters with an observable ITS plug at calving was comparable to previous observations made by Meaney (1977). The number of cows and quarters, however, was quite small in that study and there was no mention whether the study was conducted in one or many farms. According to his observations,

a plug was observable around 3 d before calving by x-ray in 32/38 (84%) of quarters. In another experiment, they reported a proportion of 93% of quarters (13 out of 14 quarters x-rayed once a week during the dry period) that kept the sealant in place for the complete dry period duration. Note that the remaining quarter kept the sealant for 11 wk out of 14 wk. Moreover, none of those 14 quarters experienced NIMI during the dry period, despite quarters being dipped into a bacteriological culture of *Staphylococcus aureus* once a week during the dry period.

In our study, in 4 out of the 6 studied dairy farms, a proportion of or all cows calved in a maternity pen

(vs. in a tie stall). Therefore, cows may have been in contact with calves after calving for some time. We can hypothesize that suckling could have played a role on the probability of observing an ITS plug at first milking. In fact, our producers confirmed that suckling by the calf could occur on some occasions, especially for those cows that calved during the night. Unfortunately, the information on suckling or on calving time were not recorded. However, one of the 2 herds with the

lowest proportion of ITS plugs present at calving was a freestall herd where cows calved in a tiestall barn. Considering these findings, factors other than suckling by the calf needs to be identified. These may include genetic characteristics of cows, the size and conformation of the teat, and so on. The relatively small associations (odds ratio of 1.4 for NIMI and 1.3 for NIMI_{env}) observed between presence or not of an observable ITS plug and odds of NIMI suggest that cows were possibly

Table 4. Results from the generalized negative Poisson mixed models used to model effect of various predictors on number of days of persistency of internal teat sealant (ITS) residues in milk after calving in 441 quarters with an observable ITS plug at calving

Parameter	Estimate	SE	Incidence ratio	95% CI	<i>P</i> -value ¹
(1) Parity					
Intercept	1.367	0.105			
Parity					<0.01
2	Reference	Reference	Reference	Reference	
3	−0.066	0.081	0.94	0.80, 1.1	
≥4	0.167	0.069	1.2	1.0, 1.4	
Herd variance	0.048	0.035			
Cow variance	0.046	0.014			
(2) Milk production pre-dry					
Intercept ²	1.395	0.118			
Milk production pre-dry ³	0.008	0.021	1.0	0.97, 1.1	0.71
Herd variance	0.051	0.038			
Cow variance	0.054	0.015			
(3) Milk production early lactation					
Intercept ²	1.448	0.200			
Milk production early lactation ³	−0.005	0.025	1.0	0.95, 1.0	0.85
Herd variance	0.051	0.038			
Cow variance	0.057	0.016			
(4) Treatment at dry-off					
Intercept	1.375	0.107			
Treatment					0.26
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	−0.068	0.061	0.093	0.83, 1.1	
Herd variance	0.050	0.037			
Cow variance	0.052	0.015			
(5) Dry period duration					
Intercept ⁴	1.422	0.120			
Dry period duration ⁵	−0.002	0.023	1.0	0.95, 1.0	0.94
Herd variance	0.051	0.038			
Cow variance	0.054	0.015			
(6) Quarter position					
Intercept	1.435	0.103			
Quarter position					0.43
Front	Reference	Reference	Reference	Reference	
Rear	0.036	0.046	1.0	0.95, 1.1	
Herd variance	0.051	0.038			
Cow variance	0.053	0.014			
(7) ITS residues visualization method					
Intercept	1.325	0.114			
Method					0.16
On floor	Reference	Reference	Reference	Reference	
Filter cup	0.280	0.197	1.3	0.90, 2.0	
Herd variance	0.042	0.034			
Cow variance	0.053	0.015			

¹Joint *P*-value for the group of predictors (type III test).

²Intercept for a cow producing 10 kg/d of milk.

³Effect for a 5 kg/d increase in milk production.

⁴Intercept for a cow with a 35-d dry period.

⁵Effect for a 7-d increase in dry period duration.

still protected against NIMI during most of the dry period. Therefore, we can hypothesize that loss of the plug occurred closely around calving because of suckling or another reason (e.g., milk hydrostatic pressure).

In our study, we hypothesized that the milk production level at drying-off would possibly affect the persistence of ITS plug, as the hydrostatic pressure due to milk production could possibly lead to the expulsion of the sealant. However, we found no evidence of association between cow-level milk production at drying

off and the presence of an ITS plug after calving. This observation was in accordance with Williamson (2001) who reported that the leaking of milk was not associated with the expulsion of the teat sealant at drying off. Moreover, we did observe a higher retention rate in rear quarters, which typically produce more milk than front quarters. In fact, ITS plug was present for 86% of rear quarters and for 80% of front quarters.

Another possibility for explaining absence of the ITS plug at first milking is postinfusion dispersion of

Table 5. Results from the generalized negative binomial mixed models used to model effect of various predictors on number of days of persistency of internal teat sealant (ITS) residues in milk after calving in 90 quarters without an observable ITS plug at calving

Parameter	Estimate	SE	Incidence ratio	95% CI	<i>P</i> -value ¹
(1) Parity					
Intercept	−0.850	0.545			
Parity					<0.01
2	Reference	Reference	Reference	Reference	
3	−0.259	0.615	0.77	0.2, 3.4	
≥4	1.808	0.628	6.1	1.3, 27.7	
Herd variance	0.609	0.816			
Cow variance	1.223	0.522			
(2) Milk production pre-dry					
Intercept ²	0.687	0.710			
Milk production pre-dry ³	−0.490	0.275	0.61	0.35, 1.1	0.08
Herd variance	0.003	0.543			
Cow variance	1.741	0.716			
(3) Milk production, early lactation					
Intercept ²	−2.754	1.662			
Milk production, early lactation ³	0.317	0.230	1.4	0.87, 2.2	0.17
Herd variance	0.915	1.271			
Cow variance	1.490	0.611			
(4) Treatment at dry-off					
Intercept	−0.404	0.522			
Treatment					0.48
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.265	0.370	1.3	0.62, 2.7	
Herd variance	0.597	1.018			
Cow variance	1.638	0.637			
(5) Dry period duration					
Intercept ⁴	−2.126	0.821			
Dry period duration ⁵	0.520	0.200	1.7	1.1, 2.5	0.01
Herd variance	0.955	1.082			
Cow variance	1.214	0.518			
(6) Quarter position					
Intercept	−0.307	0.492			
Quarter position					0.03
Front	Reference	Reference	Reference	Reference	
Rear	0.465	0.202	1.6	1.1, 2.4	
Herd variance	0.709	1.077			
Cow variance	1.527	0.601			
(7) ITS residue visualization method					
Intercept	−0.711	0.637			
Method					0.78
On floor	Reference	Reference	Reference	Reference	
Filter cup	0.433	1.574	1.5	0.07, 36.2	
Herd variance	1.196	1.842			
Cow variance	1.594	0.627			

¹Joint *P*-value for the group of predictors (type III test).

²Intercept for a cow producing 10 kg of milk per day.

³Effect for a 5 kg/d increase in milk production.

⁴Intercept for a cow with a 35-d dry period.

⁵Effect for a 7-d increase in dry period duration.

Table 6. Results from the generalized logistic mixed models used to model effect of having an observable internal teat sealant (ITS) plug at calving on odds of acquiring a new intramammary infection (NIMI) or a NIMI caused specifically by an environmental pathogen (NIMI_{env}) during the dry period

Parameter	Estimate	SE	Odds ratio	95% CI	<i>P</i> -value ¹
(1) NIMI					
Intercept	−1.256	0.344			
ITS plug at calving					0.33
Present	Reference	Reference	Reference	Reference	
Absent	0.362	0.373	1.4	0.7, 3.0	
Treatment ²					<0.01
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.885	0.276	2.4	1.4, 4.2	
Herd variance	0.405	0.369			
Cow variance	0.273	0.285			
(2) NIMI _{env}					
Intercept	−1.655	0.408			
ITS plug at calving					0.54
Present	Reference	Reference	Reference	Reference	
Absent	0.268	0.435	1.3	0.56, 3.1	
Treatment ³					<0.01
Antimicrobials + ITS	Reference	Reference	Reference	Reference	
ITS only	0.853	0.313	2.4	1.3, 4.3	
Herd variance	0.619	0.532			
Cow variance	0.073	0.339			

¹Joint *P*-value for the group of predictors (type III test).

²Treatment at dry-off was retained as a confounder of the relationship between presence of an observable ITS plug at calving and odds of acquiring a NIMI over the dry period (change in odds ratio: 15%).

³Treatment at dry-off was retained as a confounder of the relationship between presence of an observable ITS plug at calving and odds of acquiring a NIMI_{env} over the dry period (change in odds ratio: 11%).

the product in the gland cistern. This possibility was observed by Meaney (1977) and Bradley et al. (2010). Such an event could explain quarters without ITS plug at first milking and could possibly also increase persistency of ITS residues in milk in early lactation. However, we observed a reverse scenario. Quarters without ITS plug at calving had lower persistence of observable residues. Thus, our study would suggest that postinfusion dispersion, if it happens, would not increase number of days of residues after calving. In the current study, administration of ITS in conjunction with an IMM antimicrobial resulted in lower retention rates. Whether, the administration of these antimicrobials could hamper formation or retention of the plug, or promote dispersion of the product in the gland is possibly worth investigating because these products are often used together. The same question was raised by Bradley et al. (2010). The later authors hypothesized that the miscibility of an ITS and an oil-based antimicrobial may modify the viscosity of the ITS and consequently affect the ability of plug formation. Thus, they come up with a proposition for using a water-based antimicrobial when an antimicrobial is used in combination with an ITS.

We also considered that the duration of the dry period may influence the prevalence of quarters without an ITS plug at calving. However, no significant association was observed between dry period length and

odds of having an observable ITS plug at first milking. Woolford et al. (1998) reported that the sealant material was still present at the base of the teat in 19 quarters x-rayed after 100 d of the dry period. In the current study, all cows had dry period length <75 d, thus possibly not long enough to affect ITS retention.

Persistency of ITS Residues in Milk After Calving

Meaney (1977) reported on presence of ITS residues in milk after calving during the first 5 d of lactation. In the Meaney (1977) study, ITS residues were observed with a level <2.5 µg of residues/mL of composite milk at 10 DIM. On d 5, no residues were present in 26/40 (60%) of samples, and ≤5 µg of residues/mL of bulk milk in 13 samples (32.5%), and 5.1 to 16.7 µg of residues/mL in 1 sample (2.5%). Similarly, in our study, 75% of quarters had expelled the last ITS residues by 5 DIM. For some quarters, however, residues were observed up to 12 DIM. Our results on persistency of ITS residues are also similar to those of Bhutto et al. (2011). These authors report that the main part of the sealant is removed at first milking and that the remaining part is observed in milk during the subsequent milkings. However, in this later study, the exact excretion duration was not reported. These results differ from another study (Berry and Hillerton, 2002a) that reported ITS residues in milk up to 3 wk in some quarters. However,

these authors noted that none of dairy producers involved in the study reported that residue persistency was a serious concern.

Although we were able to highlight some statistically significant risk factors explaining persistency of residues following calving, observed differences were often relatively small and, perhaps, not very relevant from a clinical standpoint. For instance, 2 of the risk factors (dry period duration and quarter position) affecting ITS persistency in quarters for which the ITS plug could not be observed at first milking yielded differences in residues excretion <1 d. Parity, however, appeared to affect residue persistency more substantially, with >1 additional day of excretion for quarters of cows ≥ 4 th lactation. Data from this study indicate a higher probability of postinfusion dispersion of the product in the gland cistern for ≥ 4 th lactation. The reasons for this are unclear, but it may be due to the teat conformation in older cows, the higher hydrostatic milk pressure, or other unknown factors.

In the current study, due to the relatively large sample size, x-ray images illustrating location and persistence of ITS plug during the dry period and after calving could not be collected as was done in other studies (Meaney, 1977; Woolford et al., 1998). Further studies will be needed to confirm our results on the presence of an observable ITS plug at first milking, on ITS residues excretion during the early lactation, and on the effect of cow parity on ITS plug retention and residue excretion. For instance, in future studies, repeated ultrasound exams during the dry period and the early lactation could possibly be used to help understand ITS plug formation, retention, and excretion dynamic during these time periods.

CONCLUSIONS

Results from our study revealed that an ITS plug was present until first milking after calving for 83% of quarters and ITS residues could be observed in milk up to 12 DIM. There was no evidence that quarters without ITS plug at first milking after calving were at higher risk of NIMI or NIMI_{env} during the dry period.

ACKNOWLEDGMENTS

This research was supported by Agriculture and Agri-Food Canada, and by additional contributions from Dairy Farmers of Canada, the Canadian Dairy Network, and the Canadian Dairy Commission under the Agri-Science Clusters Initiative, through the Canadian Bovine Mastitis and Milk Quality Research Network research program, by Zoetis, and by one of

the authors (Dufour) Natural Sciences and Engineering Research Council of Canada (NSERC)-Discovery grant funds (RGPIN/435637-2013). The first author was also supported by the NSERC-Collaborative Research and Training Experience Program (CRE-ATE) in Milk Quality program. As per the research agreement, aside from providing financial support, the funders have no role in the design and conduct of the studies, data collection and analysis, or interpretation of the data. Researchers maintain independence in conducting their studies, own their data, and report the outcomes regardless of the results. The decision to publish the findings rests solely with the researchers. We are grateful to all participating producers for their cooperation, Roxanne Mandeville (Université de Montréal, St-Hyacinthe, Québec, Canada) for her support as animal health technician, and Natasha Robinson and Marguerite Cameron (both at the University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada) for their laboratory work.

REFERENCES

- Berry, E. A., and J. E. Hillerton. 2002a. The effect of an intramammary teat seal on new intramammary infections. *J. Dairy Sci.* 85:2512–2520.
- Berry, E. A., and J. E. Hillerton. 2002b. The effect of selective dry cow treatment on new intramammary infections. *J. Dairy Sci.* 85:112–121.
- Bhutto, A. L., R. D. Murray, and Z. Woldehiwet. 2011. The effect of dry cow therapy and internal teat-sealant on intra-mammary infections during subsequent lactation. *Res. Vet. Sci.* 90:316–320.
- Bradley, A. 2002. Bovine mastitis: An evolving disease. *Vet. J.* 164:116–128.
- Bradley, A. J., J. E. Breen, B. Payne, P. Williams, and M. J. Green. 2010. The use of a cephalonium containing dry cow therapy and an internal teat sealant, both alone and in combination. *J. Dairy Sci.* 93:1566–1577.
- Cameron, M., H. W. Barkema, J. De Buck, S. De Vliegher, M. Chaffer, J. Lewis, and G. P. Keefe. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. *J. Dairy Sci.* 100:2137–2147.
- CBMQRN. 2010. Administration technique of intramammary treatment in dairy cows [Online]. Accessed Dec. 14, 2017. http://www.medvet.umontreal.ca/rcrm/dynamiques/PDF_AN/Toolbox/Factsheets/AdministrationTreatmentPro.pdf.
- Dingwell, R. T., D. F. Kelton, and K. E. Leslie. 2003. Management of the dry cow in control of peripartum disease and mastitis. *Vet. Clin. North Am. Food Anim. Pract.* 19:235–265.
- Dingwell, R. T., K. E. Leslie, Y. H. Schukken, J. M. Sargeant, L. L. Timms, T. F. Duffield, G. P. Keefe, D. F. Kelton, K. D. Lissimore, and J. Conklin. 2004. Association of cow and quarter-level factors at drying-off with new intramammary infections during the dry period. *Prev. Vet. Med.* 63:75–89.
- Godden, S., P. Rapnicki, S. Stewart, J. Fetrow, A. Johnson, R. Bey, and R. Farnsworth. 2003. 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.* 86:3899–3911.
- Green, M. J., L. E. Green, G. F. Medley, Y. H. Schukken, and A. J. Bradley. 2002. Influence of dry period bacterial intramammary

- infection on clinical mastitis in dairy cows. *J. Dairy Sci.* 85:2589–2599.
- Halasa, T., O. Østerås, H. Hogeveen, T. van Werven, and M. Nielsen. 2009. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J. Dairy Sci.* 92:3134–3149.
- Huxley, J. N., M. J. Greent, L. E. Green, and A. J. Bradley. 2002. Evaluation of the efficacy of an internal teat sealer during the dry period. *J. Dairy Sci.* 85:551–561.
- Meaney, W. J. 1977. Effect of a dry period teat seal on bovine udder infection. *Isr. J. Agric. Res.* 16:293–299.
- Mickey, R. M., and S. Greenland. 1989. The impact of confounder selection criteria on effect estimation. *Am. J. Epidemiol.* 129:125–137.
- National Mastitis Council. 2017a. Sample collection and handling. Chapter 1 in *Laboratory Handbook on Bovine Mastitis*. 3rd ed. National Mastitis Council, New Prague, MN.
- National Mastitis Council. 2017b. *Laboratory Handbook on Bovine Mastitis*. National Mastitis Council Inc., New Prague, MN.
- Sanford, C. J., G. P. Keefe, I. R. Dohoo, K. E. Leslie, R. T. Dingwell, L. DesCoteaux, and H. W. Barkema. 2006. Efficacy of using an internal teat sealer to prevent new intramammary infections in nonlactating dairy cattle. *J. Am. Vet. Med. Assoc.* 228:1565–1573.
- Smith, K. L., D. A. Todhunter, and P. S. Schoenberger. 1985. Environmental pathogens and intramammary infection during the dry period. *J. Dairy Sci.* 68:402–417.
- Vuong, Q. H. 1989. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica* 57:307–333.
- Williamson, J. 2001. Strategies for protecting the teat at dry-off. The National Mastitis Council 40th Annual Meeting, February 11–14, 2001 Reno, Nevada. National Mastitis Council, Verona, WI, 88 - 94.
- Williamson, J. H., M. W. Woolford, and A. M. Day. 1995. The prophylactic effect of a dry-cow antibiotic against *Streptococcus uberis*. *N. Z. Vet. J.* 43:228–234.
- Woolford, M. W., J. H. Williamson, A. M. Day, and P. J. A. Copeman. 1998. The prophylactic effect of a teat sealer on bovine mastitis during the dry period and the following lactation. *N. Z. Vet. J.* 46:12–19.