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Effect of incomplete milking during the first 5 days in milk on udder and reproductive tract health: Results from a randomized controlled trial

C. Krug,*† P.-A. Morin,‡ P. Lacasse,†§ J.-P. Roy,†‡ J. Dubuc,‡ and S. Dufour*†[1]
*Département de Pathologie et Microbiologie,
†Canadian Bovine Mastitis and Milk Quality Research Network, and
‡Département de Sciences Cliniques, Faculté de Médecine Vétérinaire, Université de Montréal, 3200 Rue Sicotte, St-Hyacinthe, Québec, J2S 2M2, Canada
§Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, 2000 College, Sherbrooke, Québec, J1M 0C8, Canada

ABSTRACT

The aim of this study was to investigate the effect of an incomplete milking on risk of mastitis and reproductive tract disease. Multiparous dairy cows (n = 878) from 13 commercial herds were enrolled in a randomized controlled trial. Cows were randomly assigned to either a control (milked conventionally) or a treatment group, which consisted of an incomplete milking (10–14 L of milk collected/d) from 1 to 5 d in milk (DIM). Quarter milk samples were collected at approximately 11 and 18 DIM to measure somatic cell count (SCC). Quarters were considered negative for intramammary infection if SCC was <100,000 cells/mL and positive if SCC was ≥200,000 cells/mL. To calculate intramammary infection incidence, negative quarters of the initial samples collected were tested again 1 wk later. This was done to deter incidence of positive quarters. To calculate elimination rate, positive quarters were tested again 1 wk later to detect mastitis elimination. Farmers recorded clinical mastitis events. Cows were also examined at approximately 35 DIM with a Metricheck device (Simcro, Hamilton, New Zealand) for detection of purulent vaginal discharge (PVD) and with an endometrial cytobrush for presence of leukocytes [endometrial cytology for smear (ENDO) and for leukocyte esterase test (LE)]. A threshold ≥3 was used to define a positive PVD or LE test, whereas a polymorphonuclear cell count ≥6% was used to define a positive ENDO. Five generalized mixed models with cow or herd as random intercepts were used to determine the effects of incomplete milking on odds of new intramammary infection, odds of intramammary infection elimination, and odds of a positive PVD, LE, or ENDO status. To investigate time until first clinical mastitis event, a Cox model with a herd frailty term was used. The odds of new intramammary infection and intramammary infection elimination for incompletely milked cows were 0.90 [95% confidence interval (CI): 0.49, 1.7] and 2.9 (95% CI: 1.4, 6.0) times those of conventionally milked cows, respectively. The hazard of clinical mastitis in incompletely milked cows was 0.96 (95% CI: 0.59, 1.6) times that of conventionally milked cows. The odds of PVD, LE, and ENDO for incompletely milked cows were 1.4 (95% CI: 0.89, 2.1), 1.3 (95% CI: 0.88, 1.8), and 1.2 (95% CI: 0.81, 1.7) times those of conventionally milked cows. These results suggest that incomplete milking during the first 5 DIM increases the odds of a decrease in SCC from 11 to 18 DIM but does not affect odds of increase in SCC in the same period. The incomplete milking had no effect on clinical mastitis incidence in the first 90 DIM or on reproductive tract health at 35 DIM.

Key words: dairy cow, incomplete milking, mastitis, reproductive tract disease

INTRODUCTION

Dairy cows produce a large quantity of milk very quickly after parturition, and their dietary intake in early lactation is not sufficient to meet their energy requirements, which leads to mobilization of body reserves and a negative energy balance (**NEB**; Bertoni et al., 2009). This is especially true for multiparous cows, which have a very steep increase in milk yield early in lactation (Wathes et al., 2007). The resulting NEB can lead to several negative metabolic and health issues in periparturient dairy cows, including increased susceptibility to infectious diseases such as mastitis (Suriyasathaporn et al., 2000; Holtenius et al., 2004) and metritis (Reist et al., 2003; Hammon et al., 2006; Huzzey et al., 2007).

Elevated blood concentrations of nonesterified fatty acids (**NEFA**) and ketone bodies (hyperketonemia) are commonly used indices for the presence of NEB

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[1] Corresponding author: simon.dufour@umontreal.ca

because they are related to increased mobilization of fat. Several studies have shown an association between elevation of these metabolites and immunosuppression (Lacetera et al., 2005; Mulligan and Doherty, 2008; Ster et al., 2012). Nonesterified fatty acids appear to exert a direct negative effect on the immune system, but the relationship between the presence of ketone bodies and decreased immune function appears to be attributable to the link between ketone bodies and NEFA (Hegardt, 1999; Drackley et al., 2001; Ster et al., 2012). Decreasing the cow's energetic demand during the first days postpartum might improve resistance to infection by decreasing the release of NEFA into the blood circulation (Carbonneau et al., 2012; Morin et al., 2018).

Because NEB is associated with immunosuppression, we hypothesized that preventing NEB by milking dairy cows incompletely in early lactation would result in a reduction of the subsequent risk of mastitis and reproductive tract diseases. Therefore, the aim of this study was to use the large data set generated by Morin et al. (2018) and Krug et al. (2018), which was a randomized controlled trial (**RCT**), to evaluate the effect of incomplete milking during the first 5 DIM on mastitis and reproductive tract disease. Using the previous studies, we demonstrated that incomplete milking for the first 5 DIM leads to decreased odds of experiencing hyperketonemia through 17 DIM (Morin et al., 2018) without subsequent effects on milk yield and composition throughout the lactation (Krug et al., 2018). Our specific objectives for the current study were to measure, on that same population of cows, the effects of incomplete milking on (1) incidence of IMI, (2) IMI elimination rate, (3) time until first clinical mastitis event, (4) incidence risk of clinical mastitis in the first 90 DIM, (5) prevalence of purulent vaginal discharge (**PVD**), (6) prevalence of cytological endometritis (**ENDO**), and (7) prevalence of leukocyte esterase endometritis (**LE**).

MATERIALS AND METHODS

Sample Size Calculations

The original study was designed to investigate the effect of incomplete milking on ketonemia and odds of hyperketonemia (Morin et al., 2018), odds of infectious diseases, fertility, culling hazard, and milk production (Krug et al., 2018). Sample size calculation was computed for all of these different outcomes. Odds of hyperketonemia was the outcome requiring the largest sample size (no./group = 400), and therefore it determined the number of animals used in the RCT described in this study. Nevertheless, using the POWER procedure in SAS 9.4 (SAS Institute Inc., Cary, NC), we determined the minimal differences that could be detected for each of the outcomes examined in this experiment. For these calculations, we used the predetermined sample size of 400 animals per group, a 95% confidence level, 80% power, and various udder health and reproductive tract health parameters provided by the previously published literature (see Table 1). Moreover, we used the package epiR 0.9-93 (Stevenson et al., 2012) from RStudio 1.1.383 (R Core Team, 2013) to compute sample size and power calculations for time to clinical mastitis (survival analysis). Results from the sample size and power calculations are presented in Table 1. The minimal detectable clinical mastitis hazard ratio that could be detected between groups was estimated at 1.2.

Table 1. Sample size and power calculations for evaluating the effect of an incomplete milking during the first 5 DIM on udder and reproductive tract health using a 95% confidence level and 80% power

Outcome of interest	No./group	Expected prevalence or incidence in control animals (%)	Minimal detectable difference between groups (% points)
Udder health			
New IMI	1,440 quarters[1]	3.5/quarter-week (Dufour and Dohoo, 2013)	±1.7
IMI elimination	160 quarters[2]	15, 30, 50[3]	±14, ±16, ±16
Reproductive tract health			
Purulent vaginal discharge	400 cows	15 (Dubuc et al., 2010a)	±6.5
Leukocyte esterase test	400 cows	10 (Denis-Robichaud, 2013)	±5.5
Endometrial cytology	400 cows	20 (Dubuc et al., 2010b)	±7.5

[1]According to the Canadian Bovine Mastitis and Milk Quality Research Network (St-Hyacinthe, QC, Canada; Simon Dufour, personal communication), 10% out of 3,000 quarter-milk samples had ≥200,000 cells/mL at wk 2 after calving. Therefore, we would expect to have 1,440 quarters at risk of new IMI out of a total of 1,600 (4 quarters × 400 cows).

[2]For the same reasons mentioned in footnote 1, we would expect to have 160 quarters at risk of IMI elimination out of a total of 1,600 (4 quarters × 400 cows).

[3]We did not find references on IMI elimination rates. Therefore, we reported minimal detectable differences for 3 different scenarios.