Short communication: Relationship between herd intramammary infection incidence and elimination rate during the dry period

S. Dufour*†¹ and I. R. Dohoo†‡

*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, 3200 rue Sicotte, C.P. 5000, St-Hyacinthe, Quebec, J2S 7C6, Canada

†Canadian Bovine Mastitis Research Network, C.P. 5000, St-Hyacinthe, Quebec, J2S 7C6, Canada

‡Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, 550 University Ave, Charlottetown, PEI, C1A 4P3, Canada

ABSTRACT

The objective of the study was to explore the relationship between herd dry period (DP) intramammary infection (IMI) incidence and elimination rates. A cohort of 91 Canadian dairy herds was recruited and followed in 2007 and 2008. Universal dry cow therapy was widely adopted among the participating herds. At the beginning of years 2007 and 2008, a sample of 15 cows was selected in each herd. A series of quarter-milk samples consisting of 2 predry and 2 postcalving samples were collected on each quarter of these 30 cows. Milk samples were analyzed using routine bacteriological milk culture, and predry and postcalving IMI statuses of these quarters were established using parallel interpretation of the 2 predry and 2 postcalving tests, respectively. Intramammary infection status was defined as IMI by *Staphylococcus aureus*, streptococci other than *Streptococcus agalactiae*, coagulase-negative staphylococci (CNS), or *Corynebacterium* spp. Incidence and elimination rates of DP IMI were computed for each herd. Lowess curves and linear regression were used to investigate the association between DP IMI incidence and elimination rates. Significant negative associations were found between incidence and elimination rates of *Staph. aureus* and CNS. The relationship between incidence and elimination rates was nonlinear for CNS, with a relatively strong negative relationship between DP IMI incidence and elimination rates for herds with relatively low DP IMI incidence (<0.10 new IMI/quarter-month). For herds with higher DP IMI incidence (≥ 0.10 new IMI/quarter-month), a weaker negative relationship was observed between rates. No significant associations could be seen between DP incidence and elimination rates of streptococci other than *Strep. agalactiae* and of *Corynebacterium* spp. These results suggest that, conversely to the general belief,

acquisition of new IMI and elimination of existing IMI during the DP may be driven, at least for staphylococci, by common mechanisms. The use of management strategies that would lead to the selection of pathogens less well adapted to the host could explain the associations observed between acquisition and elimination of IMI over the DP.

Key words: dry period, intramammary infection, incidence, elimination

Short Communication

The dry period (DP) is recognized as a key period for acquisition and elimination of IMI (Todhunter et al., 1991; Bradley and Green, 2000; Green et al., 2002). Over the years, many recommendations have been made to prevent acquisition of new IMI (NIMI) and to increase elimination of existing IMI during the DP (NMC, 2012). The use of universal dry cow therapy (DCT; i.e., antibiotic administered to all quarters of all cows at drying off), for instance, has been proposed as a means to achieve both of these objectives (Halasa et al., 2009a,b). Some researchers have suggested that preventing NIMI and eliminating existing IMI over the DP are driven by relatively different mechanisms and would, therefore, require different decisions in term of management strategies (Green et al., 2008). Eliminating existing IMI, for instance, would be mainly driven by efficacy of a cure, whereas preventing NIMI would be driven mainly by efficacy of protection against pathogens resulting from medical interventions, by the level of exposure to pathogens, and by factors that affect a cow's susceptibility to pathogens (Green et al., 2008).

In the literature, little can be found to confirm the relative independence between herd IMI incidence and elimination rates during the DP. The relatively large sampling scheme that would be required to study the relationship between herd DP IMI incidence and elimination rates has been a major obstacle impeding studies on this matter. To obtain valid and accurate estimates of a herd DP IMI incidence and elimination

Received July 3, 2012.

Accepted November 11, 2012.

¹Corresponding author: simon.dufour@umontreal.ca

rates, for instance, most often predry and postcalving milk samples would have to be collected on a relatively large sample of quarters or cows. Furthermore, to compare herd IMI incidence and elimination rates, valid estimates of these rates would have to be computed for a relatively large sample of herds. The optimization of research funds resulting from the creation of the Canadian Bovine Mastitis Research Network (CBMRN), however, has made possible such a wide-scale longitudinal study (Reyher et al., 2011). The objective of the current study was to use the data generated on a National Cohort of Dairy Farms (NCDF) assembled by the CBMRN to evaluate the relationship between herd IMI incidence and elimination rates over the DP.

The NCDF, a cohort of 91 Canadian dairy farms, was recruited and followed for 2 yr (2007–2008). A thorough description of the selection process can be found in Reyher et al. (2011). Herds were selected in 4 regions of Canada to achieve a uniform distribution among 3 strata of bulk milk SCC and to reflect the regional distribution of housing systems. In each herd in 2007 and again at the beginning of 2008, the first 15 cows to be dried off and that were expected to remain in the herd following calving were recruited. Quarter milk samples were collected on the 30 selected cows between 4 and 2 wk before dry-off, between 2 wk and dry-off, within 24 h of calving, and from 1 to 2 wk after calving. Milk samples were frozen and later thawed and cultured, and the phenotypically different types of colonies were enumerated and speciated using recommended bacteriological procedures (Hogan et al., 1999). Briefly, 10 μ L of milk was streaked onto blood and MacConkey bi-plates and aerobically incubated at 35°C for 24 h. Colonies were enumerated and speciated after 24 h and then reincubated for an additional 24 h and re-evaluated. Unless *Staphylococcus aureus* or *Streptococcus agalactiae* were identified, milk samples with ≥ 3 phenotypically different species were considered contaminated and uninformative of the quarter IMI status. Milk samples were considered positive for *Staph. aureus* or to streptococci other than *Strep. agalactiae* when ≥ 100 cfu/mL of milk of these organisms was found. Milk samples were considered positive for CNS whenever CNS were retrieved in concentrations of ≥ 200 cfu/mL; they were considered positive for *Corynebacterium* spp. when ≥ 100 cfu/mL of milk of these organisms was found. These definitions were based on the recommendations from Dohoo et al. (2011). To define quarter IMI status at drying off and at calving, parallel interpretations of the 2 predry and the 2 postcalving samples, respectively, were used (i.e., an IMI by a specific organism or group of organisms was deemed to be present whenever ≥ 1 milk sample was positive for this organism or group of organisms). Using

these predry and postcalving IMI statuses, each herd's number of NIMI, eliminated IMI, and days at risk were calculated, and herd incidence and elimination rates (incidence and elimination density rates) computed as described in Dufour and Dohoo (2012). Descriptive analyses were conducted and the herds' incidence and elimination rate distributions inspected. The relationship between herd IMI incidence and elimination rates of each group of organisms was visually explored using locally weighted scatterplot smoothing curve (lowess) and then modeled using linear regression with the herd IMI elimination rate as dependent variable and the herd IMI incidence rate as explanatory variable. Whenever the lowess curve indicated a potential departure from a linear relationship, quadratic and cubic incidence terms were added to the model and tested for significance (F -test, $\alpha \leq 0.05$). Finally, the effect of the herd level of subclinical mastitis as a confounder or effect modifier of the incidence-elimination rates relationship was explored. To achieve this, herd mean SCS was first added to the model and the relative change between the adjusted and nonadjusted estimates of association was computed. Herd mean SCS was deemed a significant confounder whenever a $\geq 10\%$ change was observed. Then, to evaluate the effect modifier potential of mean SCS, an interaction term between herd mean SCS and incidence rate was added to the model and tested for significance (F -test, $\alpha \leq 0.05$). Herd mean SCS was not retained as a confounder or effect modifier for any of the relationships explored and these were, therefore, deemed to be unconfounded by and constant across level of subclinical mastitis.

The NCDF herds milked 85 cows on average, with a mean 305-d milk production of 9,781 kg, and a mean dry period of 81 d (SD: 24). Among the NCDF herds, 88% used universal DCT at drying off. Over the study period, 10,083 quarter series were collected and, on average, 111 quarter series (SD: 34) were available per herd to compute the incidence and elimination rates. No *Strep. agalactiae* were retrieved from predry or postcalving NCDF herd milk samples. In addition, because of the low prevalence of IMI at drying off observed for many pathogens [D. Haines (Université de Montréal, St-Hyacinthe, QC, Canada), A. M. Elmoslemany (University of Prince Edward Island, Charlottetown, PE, Canada), H. Stryhn (University of Prince Edward Island), H. W. Barkema (University of Calgary, Calgary, AB, Canada), G. Keefe (University of Prince Edward Island), K. Leslie (University of Guelph, Guelph, ON, Canada), D. Kelton (University of Guelph), D. T. Scholl (South Dakota State University, Brookings), and I. R. Dohoo, unpublished data], elimination rates for specific pathogens or groups of pathogens other than the ones previously described could not be com-

Table 1. Estimates of association between herd dry-period IMI incidence and elimination rates, for 2 groups of organisms and for *Staphylococcus aureus* and *Corynebacterium* spp., using a linear regression model (data obtained from a cohort of 91 Canadian dairy herds in 2007 and 2008)

Pathogen group IMI herd elimination rate ¹	Fixed effect	Estimate	95% CI	P-value
(1) <i>Staphylococcus aureus</i>	Intercept	0.747	0.602, 0.892	<0.01
	Incidence rate	-6.672	-11.96, -1.388	0.01
(2) Streptococci ²	Intercept	0.592	0.513, 0.671	<0.01
	Incidence rate	0.940	-0.835, 2.714	0.30
(3) CNS	Intercept	0.582	0.514, 0.649	<0.01
	Incidence rate	-1.372	-1.882, -0.862	<0.01
	Incidence rate quad. ³	2.684	0.157, 5.212	0.04
(4) <i>Corynebacterium</i> spp.	Intercept	0.664	0.583, 0.745	<0.01
	Incidence rate	-0.263	-2.203, 1.678	0.79

¹IMI incidence and elimination rates in new IMI (NIMI) and eliminated IMI per quarter-month, respectively.

²Streptococci other than *Strep. agalactiae*.

³Incidence centered on the mean (incidence - mean incidence [0.14 NIMI/quarter-month]) squared.

puted on a substantial number of herds. Analyses were, therefore, limited to *Staph. aureus*, streptococci other than *Strep. agalactiae*, CNS, and *Corynebacterium* spp. Distributions of the NCDF herd IMI incidence and elimination rates have been described before [Dufour and Dohoo, 2012; D. Haines (Université de Montréal, St-Hyacinthe, QC, Canada), A. M. Elmoslemany (University of Prince Edward Island, Charlottetown, PE, Canada), H. Stryhn, (University of Prince Edward Island), H. W. Barkema (University of Calgary, Calgary, AB, Canada), G. Keefe (University of Prince Edward Island), K. Leslie (University of Guelph, Guelph, ON, Canada), D. Kelton (University of Guelph), D. T. Scholl (South Dakota State University, Brookings), and I. R. Dohoo, unpublished data]. The relationship between IMI incidence and elimination rates for each group of organisms is presented in Figure 1. For *Staph. aureus* and CNS, negative relationships between incidence and elimination rates were observed. Furthermore, the IMI incidence-elimination relationship appeared to be nonlinear for CNS. Estimates of association between IMI incidence and elimination rates are presented in Table 1. A significant and negative association was observed between dry period incidence and elimination rates of *Staph. aureus* (i.e., higher elimination rates were observed in herd with lower IMI incidence). A quadratic incidence term was found to be a significant predictor of the elimination rate for the CNS analysis and was, therefore, used to model this nonlinear association. The CNS incidence rate was also found to be significantly associated with the elimination rate (Table 1). A relatively steep negative relationship was seen between CNS incidence and elimination rates, especially in herds with low IMI incidence (<0.10 NIMI/quarter-month; Figure 1). Finally, no significant associations could be seen between DP incidence and elimination rates for the streptococci other than *Strep. agalactiae* and for *Corynebacterium* spp.

These results suggest that acquisition of NIMI and elimination of existing IMI during the DP are related and, therefore, possibly driven in part by common mechanisms for staphylococci. Universal DCT is one management practice that can both increase the cure rate of existing IMI and ensure a certain protection against acquisition of NIMI during the DP (Halasa et al., 2009a,b). With the very high proportion of the NCDF herds already using DCT, however, it is very unlikely that the relationships between incidence and elimination rates observed would result solely from using or not DCT. Nevertheless, some differences between herds and in the DCT pharmaceutical products and methods of administration used could explain a part of the relationships observed. In a recent study conducted by Green et al. (2007), however, the pharmaceutical product used for DCT was not associated with the subsequent lactation clinical mastitis incidence rate, which is known to be strongly associated with DP IMI incidence (Bradley and Green, 2000). In addition, the pharmaceutical product used for DCT should not greatly influence the DP IMI elimination rates of CNS, which are known to show little resistance to antibiotic treatment (Waller et al., 2011). Particularities of the DCT strategy are therefore unlikely to explain the DP IMI incidence and elimination rate associations for CNS. It is likely that DCT had little effect on the results of the current study.

Biological mechanisms that could explain the low rate of NIMI observed in herds with a high IMI elimination rate might be mediated by increased cow resistance to IMI or reduced host-adaptation of the most commonly seen pathogens. In the current study, cow resistance may have been improved by the provision of well-balanced diets to the milking and dry cows or by controlling sources of stress from the environment of the cows. The resulting improved cow immune function would then yield both a higher risk

of eliminating an existing IMI and a lower risk of NIMI acquisition. Such a nonspecific improvement of the cow immune function, however, would also likely yield a higher risk of IMI elimination and a lower risk of NIMI acquisition for pathogens such as the streptococci other than *Strep. agalactiae* and *Corynebacterium* spp., a feature that was not observed in the current study.

Management of the dairy, on the other hand, could lead to selection of mastitis pathogens that are more or less well adapted to their host. The CNS, for instance, are a relatively heterogeneous group of pathogens with a potentially wide range of host adaptation (Taponen et al., 2008; Waller et al., 2011). It is likely that lower DP IMI elimination and higher IMI incidence rates would be found in herds where CNS species well adapted to the host are found. Similarly, in herds with a high prevalence of *Staph. aureus*, we could expect to

find one or a few predominant *Staph. aureus* strains coexisting with several less-common strains (Zadoks et al., 2000; Tenhagen et al., 2007). It has been speculated that these predominant strains would show a higher level of host adaptation and would both spread more easily within the herd and show a poorer response to treatment (Barkema et al., 2006). If this is the case, the relatively low *Staph. aureus* elimination rate observed in herds with high *Staph. aureus* incidence could, in fact, be the result of a higher proportion of better host-adapted strains in these herds. Such host-adaptation of specific species or strains is less likely to be observed for environmental mastitis pathogens, for which selection pressure would be mainly driven by environmental determinants. This could explain the lack of association between DP incidence and elimination rates for the streptococci other than *Strep. agalactiae* and *Corynebacterium* spp.

Figure 1. Scatter plots of herd dry period IMI incidence against herd dry period IMI elimination rate with lowess curve, for 2 groups of organisms and for *Staphylococcus aureus* and *Corynebacterium* spp.; data were obtained from a cohort of 91 Canadian dairy herds in 2007 and 2008. Three herds in panel (a) with a *Staphylococcus aureus* elimination rate >1.5 eliminated IMI (EIMI)/quarter-month are not presented to allow better comparison between panels (a) and (b); these 3 herds were, however, used to compute the lowess curve. NIMI = new IMI.

Finally, a certain level of bias due to misclassification of milk bacteriological culture is expected and could explain a part of the associations observed. In a recent study, a very high rate of CNS IMI acquisition was observed in the first 2 wk following calving [D. Haines (Université de Montréal, St-Hyacinthe, QC, Canada), A. M. Elmoslemany (University of Prince Edward Island, Charlottetown, PE, Canada), H. Stryhn, (University of Prince Edward Island), H. W. Barkema (University of Calgary, Calgary, AB, Canada), G. Keefe (University of Prince Edward Island), K. Leslie (University of Guelph, Guelph, ON, Canada), D. Kelton (University of Guelph), D. T. Scholl (South Dakota State University, Brookings), and I. R. Dohoo, unpublished data]. Some NIMI acquired shortly after calving may have been misclassified as infections that either persisted through the DP or that were acquired during the DP. Such misclassification would have led to a downward bias of the IMI elimination rate and to an upward bias of the IMI incidence rate. This may have contributed to the hypothetical biological association.

In conclusion, elimination and acquisition of staphylococci IMI over the DP seem to be related. In herds with lower rates of acquisition of *Staph. aureus* or CNS IMI over the DP, higher rates of elimination of these IMI were seen. Future longitudinal research exploring the effect of management strategies on the DP IMI dynamic (i.e., the incidence and elimination rates) is warranted to better understand the mechanisms leading to the synergism of IMI incidence and elimination rate seen during this critical period of the cow milking cycle.

ACKNOWLEDGMENTS

The authors thank all of the dairy producers, animal health technicians, and CBMRN regional coordinators (Herman Barkema, University of Calgary, Canada; Trevor DeVries, University of Guelph, Canada; Jean-Philippe Roy and Luc Des Côteaux, University of Montreal, Canada; and Kristen Reyher, University of Prince Edward Island, Canada) who participated in the data collection. This research was financed by the Natural Sciences and Engineering Research Council of Canada (Ottawa, ON, Canada), Alberta Milk Edmonton, AB, Canada), Dairy Farmers of New Brunswick (Sussex, New Brunswick, Canada), Dairy Farmers of Nova Scotia (Lower Truro, NS, Canada), Dairy Farmers of Ontario (Mississauga, ON, Canada) and Dairy Farmers of Prince Edward Island (Charlottetown, PE, Canada), Novalait Inc. (Quebec City, QC, Canada), Dairy Farmers of Canada (Ottawa, ON, Canada), Canadian Dairy Network (Guelph, ON, Canada), Agriculture and Agri-Food Canada (Ottawa, ON, Canada), Public Health Agency of Canada (Ottawa, ON, Canada), Technology

PEI Inc. (Charlottetown, PE, Canada), Université de Montréal (Montreal, QC, Canada) and University of Prince Edward Island (Charlottetown, PE, Canada), through the Canadian Bovine Mastitis Research Network (Saint-Hyacinthe, QC, Canada).

REFERENCES

- Barkema, H. W., Y. H. Schukken, and R. N. Zadoks. 2006. Invited review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine *Staphylococcus aureus* mastitis. *J. Dairy Sci.* 89:1877–1895.
- Bradley, A. J., and M. J. Green. 2000. A study of the incidence and significance of intramammary enterobacterial infections acquired during the dry period. *J. Dairy Sci.* 83:1957–1965.
- Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. *J. Dairy Sci.* 94:250–261.
- Dufour, S., and I. R. Dohoo. 2012. Monitoring dry period intramammary infection incidence and elimination rates using somatic cell count measurements. *J. Dairy Sci.* 95:7173–7185.
- Green, M., J. Huxley, A. Madouasse, W. Browne, G. Medley, A. Bradley, A. Biggs, J. Breen, M. Burnell, A. Hayton, J. Husband, J. Reader, J. Statham, and M. Thorne. 2008. Making good decisions on dry cow management to improve udder health—Synthesising evidence in a Bayesian framework. *Cattle Pract.* 16:200–208.
- Green, M. J., A. J. Bradley, G. F. Medley, and W. J. Browne. 2007. Cow, farm, and management factors during the dry period that determine the rate of clinical mastitis after calving. *J. Dairy Sci.* 90:3764–3776.
- 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., M. Nielsen, A. C. Whist, and O. Østerås. 2009a. Meta-analysis of dry cow management for dairy cattle. Part 2. Cure of existing intramammary infections. *J. Dairy Sci.* 92:3150–3157.
- Halasa, T., O. Østerås, H. Hogeveen, T. van Werven, and M. Nielsen. 2009b. Meta-analysis of dry cow management for dairy cattle. Part 1. Protection against new intramammary infections. *J. Dairy Sci.* 92:3134–3149.
- Hogan, J. S., R. N. Gonzalez, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, and K. L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI.
- NMC. 2012. NMC Recommended Mastitis Control Program. Accessed May 28, 2012. <http://www.nmconline.org/docs/NMCchecklistInt.pdf>.
- Reyher, K. K., S. Dufour, H. W. Barkema, L. Des Côteaux, T. J. Devries, I. R. Dohoo, G. P. Keefe, J. P. Roy, and D. T. Scholl. 2011. The National Cohort of Dairy Farms—A data collection platform for mastitis research in Canada. *J. Dairy Sci.* 94:1616–1626.
- Taponen, S., J. Björkroth, and S. Pyörälä. 2008. Coagulase-negative staphylococci isolated from bovine extramammary sites and intramammary infections in a single dairy herd. *J. Dairy Res.* 75:422–429.
- Tenhagen, B. A., N. Scheibe, B. A. Zucker, G. Koster, and W. Heuvelink. 2007. *Staphylococcus aureus* strains in primiparous and multiparous cows in six herds with a high prevalence of *Staph. aureus* intramammary infections. *J. Dairy Res.* 74:406–411.
- Todhunter, D. A., K. L. Smith, J. S. Hogan, and P. S. Schoenberger. 1991. Gram-negative bacterial infections of the mammary gland in cows. *Am. J. Vet. Res.* 52:184–188.
- Waller, K. P., A. Aspan, A. Nyman, Y. Persson, and U. G. Andersson. 2011. CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis. *Vet. Microbiol.* 152:112–116.
- Zadoks, R., W. van Leeuwen, H. Barkema, O. Sampimon, H. Verbrugh, Y. H. Schukken, and A. van Belkum. 2000. Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* 38:1931–1939.