A case-control study of *Nocardia* mastitis in Ontario dairy herds

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Abstract

A case-control study was conducted to identify milking hygiene and udder therapy factors associated with a diagnosis of *Nocardia* mastitis in dairy herds from which milk samples are submitted to the Ontario Ministry of Agriculture and Food, Veterinary Laboratory Services, Guelph laboratory. The data were collected by telephone interview from 31 case and 31 control herds.

After analytical control for confounders, blanket dry cow therapy and a dry cow antibiotic product were associated with a diagnosis of *Nocardia* mastitis in a herd, whereas another dry cow antibiotic product had a sparing effect.

In comparison to mastitis pathogens susceptible to dry cow antibiotic therapy, *Nocardia* species were isolated infrequently from milk specimens submitted to the Guelph mastitis laboratory.

Résumé

Études de cas-témoins de mastite causée par les *Nocardia* dans les troupeaux laitiers de l'Ontario

Une étude contrôlée fut menée sur des échantillons de lait soumis au ministère de l'agriculture et de l'alimentation ontarien, service de laboratoires vétérinaires, laboratoire de Guelph, afin de déterminer les facteurs impliqués dans le développement de mammite à *Nocardia* et pouvant être reliés à l'hygième lors de la traite et/ou à des thérapies de la glande mammaire. Les données ont été obtenues par interview téléphonique pour 31 cas et 31 contrôles.

Après analyse des contrôles, la thérapie de couverture et la thérapie avec un antibiotique pour vaches taries furent associées avec un diagnostic de nocardiose mammaire dans un élevage tandis qu'un autre antibiotique pour vaches taries avait un effet protecteur.

***Nocardia* fut isolé rarement des échantillons de lait soumis au laboratoire de Guelph en comparaison avec d'autres pathogènes susceptibles aux préparations d'antibiotiques pour vaches taries.**

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Introduction

Nocardia asteroides was first identified as a mammary pathogen in 1958 (1) and has remained a sporadic cause of mastitis since that time. It is a soil organism with no known geographic restrictions. Treatment is generally unsuccessful, probably because of the granulomatous lesion formed in the udder.

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Over the past several years there has been an increase in the number of *Nocardia* isolations from milk samples submitted for culture to the Ontario Ministry of Agriculture and Food (OMAF) Veterinary Laboratory Services (VLS) laboratory at Guelph. The proportions of *Nocardia* isolations from all milk submissions were 0.003, 0.02, 0.06 and 0.25 for the years 1985, 1986, 1987 and 1988, respectively (unpublished data, VLS). Veterinarians from other provinces also reported that they were isolating *Nocardia* species from milk samples more often than expected. In the past, unsanitary intramammary therapy has been linked to *Nocardia* infections (2). Other proposed explanations for the increase in the number of *Nocardia* isolations include improved diagnostic techniques, greater numbers of milk samples being examined, and an increase in the level of *Nocardia* organisms in the environment.

This study was conducted to identify milking hygiene and therapeutic factors which may be associated with *Nocardia* mastitis in Ontario dairy herds from which milk samples were submitted to the OMAF VLS laboratory. A secondary objective of the study was to determine if herds in which specific dry cow products were used were at greater risk of *Nocardia* mastitis.

Materials and methods

The herd was chosen as the unit of concern and analysis because milking management techniques are generally applied in a similar fashion to all cows within a herd. Both control and case herds were obtained from those herds submitting milk samples for culture to the OMAF VLS laboratory in Guelph between January and July 1988. When a *Nocardia* species was cultured from a milk sample, the herd of origin became a case herd. Whenever possible, the herd of origin of the next milk sample containing a mastitis pathogen other than *Nocardia* was used as a control herd. Five owners identified as having control herds chose not to participate in the study because they were too busy planting spring crops. In these five instances, replacement control herds were nonrandomly selected by convenience from milk samples submitted during the same time period. Owners of herds selected as controls were asked if *Nocardia* had been cultured previously. Additional milk cultures were not conducted in control herds.

When this study was initiated, there was great concern about the possibility of a contaminated udder therapy product. A previous telephone survey of 18 *Nocardia*-positive herds revealed that product A (neomycin and hydrocortisone) was used in 50% of them. According to the manufacturer, product A held

only 15% of the total market share for dry cow intramammary antibiotics. The minimum sample size was determined using these estimated rates and the formula (3):

$$n = [Z_\alpha(2PQ)^{1/2} - Z_\beta (P_eQ_e + P_cQ_c)^{1/2}]^2 \div (P_e - P_c)^2$$

where n = estimated sample size for each of the case and control groups.
Z_α = 1.96 (type I error, = 0.05)
Z_β = −0.84 (type II error, = 0.20)
P_e = estimated proportion of *Nocardia*-positive (case) herds exposed to product A (0.50).
P_c = estimated proportion of *Nocardia*-negative (control) herds exposed to product A (0.15).
$P = (P_e + P_c) \div 2$
$Q = 1 - P$

Thirty-one case herds and 31 control herds were included in the study. The survey questions pertained to the variables listed in Table 1. Herd milk production, milk quality measures, milking sanitation techniques, treatment procedures, and housing were the focus of the survey questions.

The data were analyzed using the SAS (4) statistical software package. The means of the normally distributed quantitative variables were compared using the Student's *t*-test. For qualitative variables, frequencies were compared using the Chi-square or Fisher's Exact tests. Odds ratios were used as measures of the strength of association. The precision of the measures of association were investigated using the 95% precision-based confidence intervals. Variables considered confounders were controlled analytically using the Mantel-Haenszel technique. A significance level of 5% was used in all analyses.

Table 1. Variables examined in the unconditional analyses

1. main BREED of cattle
2. Ontario Dairy Herd Improvement Corporation milk recording
3. milking HERD SIZE
4. MILK PER MILK COW PER DAY
5. BCA — MILK
6. BCA — FAT
7. SOMATIC CELL COUNT
8. PLATE LOOP COUNT
9. STALL type and BEDDING
10. MATERNITY PEN used
11. WHO MILKS the cows
12. WHO administers the udder treatments
13. is DRY COW ANTIBIOTIC THERAPY used
14. how are COWS SELECTED for dry cow therapy
15. DRY COW ANTIBIOTIC PRODUCT used
16. dry cow product packaging: INDIVIDUAL TUBES or BULK
17. TEAT AND PREPARATION before therapy
18. TEATS DIPPED — after treatments, premilking, postmilking
19. TEAT DIP PRODUCT AND MANUFACTURER
20. type of CONTAINER used to apply teat dip
21. DIP CHANGED how frequently
22. UDDER WASH PRODUCT and MANUFACTURER
23. udders WASHED prior to milking
24. udders DRIED prior to milking

Results

All owners of case herds agreed to participate in the study. Eighty-two percent of the owners milked Holstein cattle and 78% were enrolled in the Ontario Dairy Herd Improvement Corporation milk recording scheme. Some of the general characteristics of the participating herds are shown in Table 2. The case and control herds were similar in size, milk per cow per day, and BCA for milk and fat, although the difference in the mean BCA for milk bordered on significance ($p = 0.054$).

Three variables were associated unconditionally with a risk of *Nocardia* mastitis in a herd. They were the herd somatic cell count (SCC), the use of blanket dry cow therapy (BDCT), and the dry cow product used (Table 3). Herds with SCC of less than 500,000 cells/mL were 19.7 times more likely to experience a case of *Nocardia* mastitis. Repeating the analysis using a 200,000 cells/mL cutoff point revealed that herd SCC did not increase the risk of a *Nocardia* diagnosis in a herd. In those herds using dry cow therapy, the use of BDCT (versus selective) increased the probability 8.0 times. The risk was 6.7 times greater when one product (A) was used and was nine times lower when another product (B) (cloxacillin) was used.

Table 4 contains the results of the Mantel-Haenszel analyses. After analytical control for blanket dry cow therapy, there was no association of SCC with a *Nocardia* diagnosis in a herd. With SCC, product A, or product B controlled, BDCT had a positive association with a herd diagnosis of a *Nocardia* mastitis. When BDCT was controlled, product A remained associated with *Nocardia* mastitis diagnosis and product B retained a sparing effect.

Discussion

A desire to assist with the investigation of an actual or potential problem of economic significance to their herd probably contributed to the excellent participation of producers in the study. Conducting the survey by telephone interview was expeditious and inexpensive. Because of these features, we favored the technique for this survey even though one may question the validity of the data collected by this method rather than by personal observation of one investigator or many collaborating veterinary practitioners.

The increased number of *Nocardia* mastitis diagnoses in Ontario herds during 1987 and 1988 prompted the survey. The hearsay regarding the relationship of dry cow antibiotic product A with *Nocardia* mastitis (5) influenced the sample size determination. The sample size was sufficient to perform unconditional analyses; however, a larger sample size would have permitted more conditional analyses.

Control herd status was verified only by questioning if *Nocardia* had been diagnosed previously in the herd. Some control herds could conceivably have been case herds. More intensive verification would have required microbiological culture of bulk tank milk or composite milk samples from all individual cows in the herd. Use of the former has a risk of environmental contamination (6) and a probable lack of sensitivity, while use of the latter contains those elements plus the

Table 2. General characteristics of Ontario dairy herds participating in a *Nocardia* mastitis study

	Case herds[c]	SD	Control herds	SD
Percentage milking Holsteins	83		81	
Percentage enrolled in ODHIC[a]	87		71	
Average size of milking herd	44	31.3	53	21.0
Average milk/cow/day (L)	24	2.7	22	6.0
Average BCA[b] MILK	160	13.3	153	14.6
Average BCA FAT	161	13.0	147	36.9

[a]Ontario Dairy Herd Improvement Corporation
[b]Breed class average
[c]All differences between case and control herds are nonsignificant ($p > 0.05$)

Table 3. The association of some specific factors with the diagnosis of *Nocardia* mastitis in Ontario dairy herds

	Nocardia					
	Yes	No	Total	P	OR[a]	95% CI[b]
SCC < 500,000/mL	29	22	51			
SCC > 500,000/mL	0	7	7	0.010	19.7	1.1, 363
DCT[c]	31	26	57			
No DCT	0	5	5	0.052	13.1	0.7, 248
BDCT[d]	28	14	42			
Selective DCT	3	12	15	0.003	8.0	1.9, 33
Product A[e]	17	4	21			
Other products[f]	14	22	36	0.003	6.7	1.9, 24
Product B[g]	2	10	12			
Other products	29	16	45	0.007	0.1	0.0, 0.6

[a]Odds ratio
[b]95% precision-based confidence interval of odds ratio
[c]Dry cow therapy
[d]Blanket dry cow therapy
[e]Dry cow antibiotic product A
[f]Other dry cow antibiotic products
[g]Dry cow antibiotic product B

expense inherent in collection and culture of numerous individual samples. How frequently reculturing must be done to confirm negative status is yet another issue to consider. Some compromise could probably assure control herd status for future studies.

Bias in the estimate of the odds ratio may be present from the misclassification of control herds and from the selection of cases and controls based upon submission of milk samples to the diagnostic laboratory. The effects of selection bias and misclassification bias on measures of association have been reviewed by Kleinbaum (7) and Lilienfeld (8). An example in veterinary medicine has been presented by Martin (9). The general opinion is that odds ratios of greater than three are probably not spurious, although they may be overestimated. Although matching was not attempted, the case and control herds were similar in breed of cattle, enrolment in milk recording, herd size, and milk production.

There was a high probability of interviewer bias in this study. The person conducting interviews was aware of the rumors of contaminated udder products and the same rumors were rampant among Ontario dairymen. In addition, the interviewer was not blinded to the status of each herd because the control herd owners were asked at the very start of the interview if *Nocardia* mastitis had been diagnosed in their herds previously. Despite these shortcomings, the interviewer attempted to conduct the questioning with impartiality.

The variables examined were chosen because of a hypothesized relationship between *Nocardia* mastitis and udder therapy, environmental contamination and milking sanitation. The majority of questions pertained to techniques of antibiotic therapy and to milking management practices. Herd statistics related to breeds, cow numbers, milk production, and raw milk quality were included along with a few questions on housing. The low frequency of adoption of some techniques prevented analysis using all the variables. For example, bulk packages of dry cow antibiotic preparations were infrequently used and thus analyses of the data on the basis of individual tubes versus bulk packaging were not possible.

During interviews, owners and veterinarians had expressed some concern that low herd SCC could be associated with an increased risk of *Nocardia* mastitis. The Mantel-Haenszel analysis controlling for blanket dry cow therapy revealed that herd SCC in itself did not have a significant effect on the probability of *Nocardia* mastitis diagnosis in a herd. Blanket dry cow therapy was responsible for the significant part of the apparent association.

Table 4. The relationship of somatic cell count (SCC), blanket dry cow therapy (BDCT) and specific dry cow antibiotic products with *Nocardia* mastitis in Ontario dairy herds after analytical control for confounders using the Mantel-Haenszel procedure

Variable of interest	Control variable	χ^2_{mh} [a]	OR[b]	95% CI[c]
SCC	BDCT	4.2[d]	4.8	0.5, 46.1
BDCT	SCC	7.5	7.1	1.6, 31.3
	Product A[e]	7.1	6.9	1.6, 29.7
	Product B	13.5	9.6	2.3, 39.6
Product A	BDCT	7.0	5.9	1.4, 25.1
Product B	BDCT	12.6	0.1	0.0, 0.5

[a]Chi-square Mantel-Haenszel
[b]Odds ratio
[c]95% precision-based confidence interval of the odds ratio
[d]Not significant ($p > 0.05$), all other chi-squares are significant
[e]Product A and product B refer to dry cow antibiotic products

Table 5. Frequency of isolation of pathogens from milk submissions to the Guelph Veterinary Laboratory Services mastitis laboratory in 1988

Pathogen	Number	Frequency	SD
Hemolytic *Staphylococcus*	1116	9.1	0.26
Streptococcus agalactiae	876	7.2	0.23
Streptococcus nonagalactiae	750	6.1	0.22
Coliform sp.	221	1.8	0.12
Nocardia sp.	163	1.3	0.10
Yeast	38	0.3	0.01
Actinomyces pyogenes	32	0.3	0.01
Pseudomonas sp.	14	0.1	0.03
Pasteurella sp.	14	0.1	0.03
Other	8	0.1	0.03
No isolation	9011	73.6	0.40
Totals	12243	100.0	

After controlling for herd SCC, product A, and product B, the summary odds ratios of the association between blanket dry cow therapy and the occurrence of *Nocardia* mastitis were significant, in the same direction, and of approximately the same magnitude as the crude odds ratio. Since no significant interaction was detected, it appears that the use of blanket dry cow therapy increases the risk of *Nocardia* mastitis in the same order of magnitude independent of the products used. Similar conclusions were reached after testing the association between both dry cow antibiotic products A and B and the occurrence of *Nocardia* mastitis following control for blanket dry cow therapy.

The positive association of blanket dry cow therapy and product A and negative association of product B with *Nocardia* mastitis should be interpreted cautiously. A small sample size (31 case and 31 control herds) with 21 herds using product A and 12 herds using product B limited the detail and complexity of the analyses.

At the time of this study, the manufacturer of product A conducted exhaustive microbiological testing of their product through in-house and private laboratories. Contamination with *Nocardia* was not detected. While dry cow antibiotic therapy was the variable most consistently associated with *Nocardia* mastitis in a herd, the precise nature of this association and the pathogenesis of the mastitis is not presently understood. Experiments to investigate the pathogenesis and to evaluate any possible role of dry cow antibiotic products in *Nocardia* udder infections may be beneficial. Specific practices associated with treating dry cows were not identified by this study and some practices may also alter the risk of *Nocardia* mastitis. Refining the study variables, using a larger sample size, and repeating the case-control study in Ontario and other provinces may provide more insight into the associations identified in this study.

Mastitis pathogens susceptible to dry cow antibiotic therapy were isolated from milk specimens submitted to the Guelph mastitis laboratory more frequently than was *Nocardia* during the progress of this study (Table 5) (unpublished data, VLS). For this reason, the potential economic benefits that may be derived from the use of dry cow therapy (10) should not be abandoned in the face of the small increase in the risk of *Nocardia* mastitis.

In summary, this case-control study investigated herd milking management practices for associations with the diagnosis of *Nocardia* mastitis. Herd size, milk per cow per day, BCA milk and BCA fat were similar for case and control herds. After controlling

for blanket dry cow therapy, herd SCC was not associated with a *Nocardia* mastitis diagnosis in a herd. Blanket dry cow therapy and the use of dry cow antibiotic product A were positively associated with a *Nocardia* diagnosis in a herd. The use of dry cow product B had a sparing effect on the risk of a *Nocardia* diagnosis in a herd. Because the associations reported are based upon an observational study, further studies will be required to verify these associations.

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