PEER REVIEWED

Methods to Monitor New Intramammary Infections in Dairy Cows Using Somatic Cell Count Data

M. A. Kristula, *DVM, MS, University of Pennsylvania, New Bolton Center, Kennett Square, PA 19348*

Abstract

This report provides methods to monitor the number of new mammary infections in dairy cattle using monthly Dairy Herd Improvement somatic cell count data. The new infection rate on a monthly basis is defined as the number of cows over linear score 4 for the first time, divided by the total number of cows at risk. The cumulative analysis of days-in-milk when new infections occur allows for quantification of the highest risk period by days-in-milk for the occurrence of new infections. The methods outlined enable some statistical inferences to be made about the overall mammary infection rate, and the greatest time period of risk for new infections. This information can be used to evaluate management decisions aimed at lowering the number of new mammary infections.

Résumé

Cette présentation fournit des méthodes pour suivre le nombre de nouvelles infections mammaires chez les bovins laitiers en utilisant les données mensuelles sur le comptage des cellules somatiques provenant du programme d'amélioration des troupeaux laitiers. Le nombre de nouvelles infections sur une base mensuelle est défini comme étant le nombre de vaches dont le score linéaire est au-delà de 4 pour la première fois, divisé par le nombre total de vaches à risque. L'analyse cumulative des jours en lait lorsque les nouvelles infections ont lieu permet de déterminer la période la plus à risque en fonction des jours en lait pour l'apparition de nouvelles infections. Les méthodes décrites ici permettent de faire quelques inférences statistiques sur le taux d'infection mammaire en général et sur la période la plus à risque pour le développement de nouvelles infections. Cette information peut être utilisée pour évaluer les pratiques de gestion dont le but est de réduire le nombre de nouvelles infections mammaires.

Introduction

Mastitis is caused by microorganisms that invade the udder through the teat canal and cause inflammation, and may be clinical or subclinical. Mastitis is termed clinical mastitis when abnormal milk or mammary gland swelling is present, and subclinical when the milk and mammary gland are grossly normal, but the somatic cell count (SCC) in the gland is elevated. Elevated SCC result from the influx of leukocytes into the mammary gland in response to infection by microorganisms.

The ultimate goal of record keeping on modern dairy farms is to collect data in an appropriate form so that statistical inferences can be made regarding changes in herd performance after intervention. To make statistical inferences, observations under analysis must be independently and identically distributed with respect to disease risk. For example, although a cow can have a new infection in each of her quarters and one of her quarters may develop multiple new infections over time, a cow can contribute only one infection to a data set.[6] Similarly, when comparing two data sets, observations in each data set must be independent. The same cow cannot be included in both data sets. These assumptions are often violated when analyzing farm records, especially in herds with small numbers of cows and observations. To the extent that these assumptions are violated while analyzing farm data, false conclusions may be made regarding the effectiveness of management intervention.

Dairy herds need to measure the number of new mammary gland infections, and the time in lactation when these infections occur, in order to identify and assess management interventions aimed at preventing new infections. The number of new mammary gland infections can be measured by counting cases of clinical mastitis as they occur over time,[5] or by defining a new infection as the first time a cow's linear score is greater than 4 or 5 in a lactation.[7] The major bias in counting the clinical cases of mastitis would be accurate enumeration and identification of clinical cases by the milkers, and that the total number of clinical cases will underestimate the number of mammary glands actually infected.

Electronic somatic cell counting is widely used as an indirect method to calculate the number of new mammary gland infections. It is inexpensive, convenient and accurate.[4] Because marketable milk and quality premiums are based on somatic cell cutoff values, somatic cell counting analysis is the ideal method to monitor the new mammary infection rate.[4] The major bias in using SCC greater than a cutoff value for identifying new infections would be the likelihood of missing some infections because SCC data is only collected monthly by dairy herd improvement associations (DHIA).

Materials and Methods

The following example illustrates methods of defining and monitoring the number of new mammary gland infections on a dairy farm using DHIA monthly SCC data.

1. Proportion of cows infected for the first time each month

A new mammary gland infection is defined as the first time a cow's SCC is greater than 200,000/ml (linear score greater than 4) on a monthly DHIA test. Once a cow is defined as having had a new mammary infection, she is no longer at risk of having another mammary infection, therefore she would not be included in further denominators listing cows at risk for new mammary infections. The proportion of cows infected for the

Table 1. Proportion of cows with new mammary infections by days-in-milk.

DIM at risk[a] intervals	Cows at[b] risk	New[c] MI	New MI[d] data	New MI[e] proportion
Interval 1 <50	41	9	9/41	22.0% (11.1% - 38.0%)
Interval 2 51-100	50	3	3/50	6.0% (1.5% -17.5%)
Interval 3 101-150	25	1	1/25	4.0% (0.2% - 22.3%)
Interval 4 151-200	24	0	0/24	0% (0.4% - 17.2%)
Interval 5 201-250	17	2	2/17	11.8% (2.06% - 37.7%)
Interval 6 251-300	9	1	1/9	11.1% (0.6% - 49.3%)
Interval 7 >301	20	3	3/20	15.0% (3.9% - 38.9%)
Overall new MI	196	19	19/196	9.7% (6.1% - 14.9%)

[a]Intervals of 50 days-in-milk (DIM).
[b]Number of cows by DIM at risk for a new mammary infection (MI) in the interval.
[c]New MI. By definition these are cows with their first linear score above 4 for the current lactation.
[d]Numerators and denominators used to calculate the proportion of cows with new MI.
[e]The proportion of cows with new MI and 95% confidence intervals.[3]

first time each month is defined as the number of cows over linear score 4 for the first time, divided by the total number of cows at risk for a new mammary infection. In this example (Table 1), 284 cows have linear score data from the last DHIA monthly test, but only 196 cows have never had a linear score above 4 during this lactation. Therefore the denominator for the proportion, or the population at risk for developing a new mammary infection, is 196 cows. Since 19 of these 196 cows had a linear score above 4 for the first time this DHIA test, the proportion of cows with a new mammary gland infection is 9.7% for the test period. The 95% confidence interval[3] implies that the real percentage of cows infected for the first time in this test period is somewhere between 6.1% and 14.9%. The proportion of cows infected for the first time should be compared on a monthly basis with caution, because the data are not independent, since cows not infected this month could appear in both this month's and next month's denominator. However, evaluating the trends in the proportion of cows infected for the first time on a monthly basis will provide a baseline from which to compare over time.

The occurrence of new mammary infections can be analyzed by days-in-milk (DIM), thereby allowing for an assessment of when new infections are occurring. Utilizing current test day information and at-risk intervals of 50 DIM, the proportion of cows with new mammary infections can be calculated for each interval. Table 1 illustrates a breakdown of the mammary infection rate using intervals of 50 DIM. Any period of time could be used for an interval, and the intervals do not have to have the same number of days. However, the assumption is that the infection rate is constant in the interval. Intervals of time should be chosen to provide a reasonable number of cows in each interval. In this example (Table 1), the denominator for the first interval of less than 50 DIM includes all cows that are at risk for a new mammary infection (have not had a linear score of 4 yet). Since nine of the 41 cows at risk had a linear score above 4 for the first time, the proportion of cows infected is 22% for cows less than 50 DIM. Cows that had linear scores over 4 at dry-off and at their first DHIA test are not included in this first interval since they are assumed to be previously infected.

The numerators for the successive time intervals of risk are obtained by counting the number of cows with new infections in the particular DIM interval under consideration for the test period. The denominators for each interval of DIM are obtained by counting the total number of cows at risk for a new infection in the DIM interval under consideration.

The proportion of cows infected in each time period can be analyzed by comparing confidence intervals. Days-in-milk risk intervals without overlapping confidence intervals would be significantly different.

2. Cumulative analysis of DIM when new infections are occurring

A cumulative analysis of the DIM when new infections are occurring can be obtained by analyzing the DIM when each cow in the herd first had a linear score greater than 4. Table 2 illustrates a breakdown of the proportion of cows having a linear score greater than 4 for the first time by intervals of 50 DIM. In this example, the denominator for the first interval of less than 50 DIM is 332, which includes all of the current milking cows and cows that have been culled in the past year. Sixty three of the 332 cows had linear scores of 4 or greater for the first time when less than 50 DIM. The percentage of cows with new mammary infections in the less than 50 DIM interval is 18.9% (95% confidence interval 15.0% - 23.8%). The numerators for the successive DIM intervals of risk are obtained by counting the numbers of cows with new infections in the DIM interval under consideration for the test period. The denominators for each interval of DIM are obtained by subtracting all of the cows in the previous intervals from the total cows at risk, analogous to Kaplan-Meier survival methodology.[1] The real percentage of cows infected for a DIM risk interval is described by the 95% confidence interval.

3. Kaplan-Meier survival curves[1,2]

Kaplan-Meier survival curves are a dynamic way to measure the new mammary infection rate and the DIM when infections are occurring. The advantages of using survival curves to analyze mastitis data is that data from cows with and without mastitis are analyzed simultaneously. Cows culled before developing mastitis and cows that have not yet had mastitis are considered censored and their DIM at the time of analysis of the data are used. For cows with mastitis, their DIM when the mastitis occurred are used.

Results and Discussion

For this herd, the overall percentage of cows with new mammary infections for the test period is 9.7% (95% CI 6.1 - 14.9%; Table 1). The point estimate of the percentage of cows infected for the interval of less than 50 DIM is 22%, which is higher than the proportion of cows infected for the first time in the other DIM intervals. However, all of the confidence intervals overlap due to the small number of cows in each time interval. As the time intervals extend beyond 100 DIM, the number of cows in each interval is smaller and the confidence intervals become wider, making significance testing difficult.

The proportion of cows infected in a given time period can be compared to the same time period on a monthly basis by comparing confidence intervals. This analysis is valid if the same cow does not appear in both

Table 2. Cumulative proportion of cows with new mammary infections by days-in-milk.

DIM at risk[a] intervals	Cows at[b] risk	New[c] MI	New MI[d] data	New MI[e] rate
Inteirval 1 <50	332	63	63/332	18.9% (15.0% - 23.8%)
Interval 2 51-100	268	23	23/268	8.6% (5.6% - 12.8%)
Interval 3 101-150	233	14	14/233	6.0% (3.5% - 10.1%)
Interval 4 151-200	189	7	7/189	3.7% (1.6% - 7.8%)
Interval 5 201-250	147	13	13/147	8.8% (5.0% - 15.0%)
Interval 6 251-300	96	10	10/96	10.4% (5.4% - 18.7)
Interval 7 >300	58	14	14/58	24% (14.3% - 37.4%)

[a]Intervals of 50 days-in-milk (DIM).
[b]Number of cows by DIM at risk for a new mammary infection (MI) in the interval.
[c]New MI. By definition these are cows with their first linear score above 4 for the current lactation.
[d]Numerators and denominators used to calculate proportion of cows with new MI.
[e]The proportion of cows with new MI and 95% confidence intervals.[3]

monthly intervals to be compared. Using 30-day time intervals will assure the same cows do not appear in both monthly intervals to be compared, but may not provide enough numbers to make valid comparisons. Statistical inferences are difficult to impossible in small herds with few numbers in each interval of time. This analysis assumes the risk of a new infections is constant for any particular DIM time period.

The point estimate for the cumulative proportion of cows infected for the first time in the interval of less than 50 DIM is 18.9% (Table 2), which is higher than the point estimate for the proportion of cows infected for the first time in the other time intervals, except for the interval beyond 300 DIM. However, there is overlap of confidence intervals for the interval of less than 50 DIM, and all of the intervals greater than 200 DIM. Beyond 200 DIM, the number of cows in each interval is smaller, resulting in wider confidence intervals, which tends to bias upwardly the proportion of cows infected.

The data suggests the highest risk period for new infections in this herd is before 50 DIM; 18.9% of new mammary infections are occurring before 50 DIM. This analysis has a lot of computational momentum and since the same cows contribute to the same intervals for some time, the results should be compared on a yearly basis to make statistical inferences, and not from month to month.

The power of the Kaplan-Meier analysis is that at any particular days-in-risk an instantaneous risk of infection is calculated, and the underlying rate of infection does not need to be constant (Table 3). Therefore, time intervals of assumed constant risk (Table 2) do not need to be made. From Figure 1, qualitative assessment suggests the steepest decline of the curve, or greatest risk period for new mammary infections, are less than 50 DIM and after 300 DIM. The Kaplan-Meier estimates listed in Table 3 are for survival rates, or in this case, cows that did not get mastitis. To obtain the instantaneous rate of new mastitis infections at any DIM, the survival rate and corresponding confidence intervals are subtracted from 1. The Kaplan-Meier estimate of the new mammary infection rate by 50 DIM is 18.8% [(1-.8125)*100], with 95% confidence intervals of

Table 3. Partial table of Kaplan-Meier estimates.[2]

		95% CI[c]			
Time[a]	K-M[b]	Lower	Upper	At risk	Failed
2	.9909	.9722	.9971	331	3
4	.9819	.9601	.9918	328	3
5	.9789	.9562	.9899	325	1
6	.9728	.9498	.9858	324	2
7	.9607	.9333	.9770	322	4
8	.9577	.9296	.9747	318	1
9	.9517	.9223	.9701	317	2
11	.9426	.9115	.9630	315	3
12	.9335	.9008	.9557	312	3
13	.9275	.8938	.9508	309	2
14	.9245	.8903	.9483	307	1
15	.9215	.8868	.9458	306	1
17	.9154	.8798	.9408	305	2
18	.9124	.8764	.9383	303	1
19	.9094	.8729	.9357	302	1
20	.9033	.8661	.9306	301	2
21	.8973	.8592	.9255	299	2
22	.8912	.8524	.9203	297	2
23	.8882	.8491	.9177	295	1
25	.8852	.8457	.9151	294	1
26	.8822	.8423	.9125	293	2
28	.8792	.8389	.9099	292	1
29	.8761	.8356	.9072	291	1
31	.8731	.8322	.9046	290	1
32	.8671	.8255	.8993	289	2
35	.8610	.8189	.8940	287	2
36	.8550	.8122	.8887	285	2
37	.8489	.8056	.8833	282	2
38	.8429	.7990	.8779	280	2
41	.8368	.7924	.8725	278	2
45	.8307	.7858	.8679	276	2
46	.8277	.7825	.8643	274	1
47	.8247	.7792	.8616	273	1
48	.8186	.7727	.8561	272	2
49	.8156	.7694	.8534	270	1
50	.8125	.7661	.8506	269	1

[a]Days-in-milk. Only days 1-50 are shown in this table.
[b]Kaplan-Meier estimate of survival (KMsurvival).
Kaplan-Meier estimate of failure = 1 - KMsurvival.
[c]Confidence interval for survival (CIsurvival).
Confidence interval for failure = 1 - CIsurvival.

14.9 - 23.4%. The relatively steep decline in the curve in Figure 1 after 300 days should be noted, but interpreted with caution, as there are fewer cows in the data set beyond 300 DIM.

The Kaplan-Meier curves should be calculated and compared on an annual basis. More sophisticated modeling can be done by controlling for such factors as lactation number and season of calving.

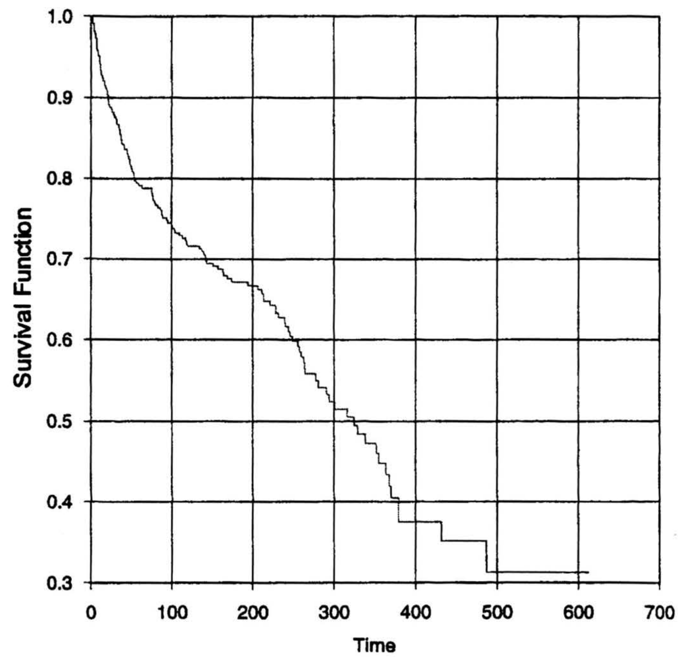

Figure 1. Survival function for Kaplan-Meier estimation.

For this herd, the major risk period for new mammary infections is in the first 50 DIM. Management changes should be directed towards the dry and recently calved cows. As management makes changes to address the high infection rate in the first 50 DIM, statistical inferences will be possible using the described methods of analysis to evaluate the success of the intervention.

Conclusions

This paper outlines methods of analyzing the number of new mammary infections in dairy herds using DHIA monthly SCC data. The basic premise used in the analysis is that once a cow is defined as having had a new mammary infection, she is no longer at risk of having another mammary infection. This definition eliminates the ambiquity of having to determine the current mammary infection status of previously high linear score cows, and ensures a population of truly non-infected cows to evaluate management changes.

Because observations of farm data to be compared are often not independently and identically distributed with respect to mastitis risk, statistical inferences are not always possible. Data should be observed for trends, especially on a month to month comparison. Statistical inferences are generally more accurate when a year to year comparison of data is made, particularly in small herds.

References

1. Allison PD: Event history analysis. A Sage University Paper Series. Series: *Quantitative Applications in the Social Sciences*. Series Number 07-046, Sage Publication, Beverly Hills, CA, 1984.
2. Egret software for analysis of biomedical and epidemiological studies. Cytel Software Corporation, Cambridge, MA, 1999.
3. Fleis JL: *Statistical methods for rates and proportions,* ed 2. New York, John Wiley and Sons, 1981, pp 15.
4. Heald CW: Practical uses and limitation of somatic cell counts. *Proc 34th Annual Meeting of the NMC,* 1995, pp 23-29.
5. Kristula M, Uhlinger C: Paper monitoring records. *Compend Contin Educ Prac Vet* 17:1520-1525, 1996.
6. Kristula M, Curtis C, Galligan D, Bartholomew R: Evaluation of a repeated measures logistic regression model to predict chronic mastitis in dairy cows. *Prev Vet Med* 14:57-68, 1992.
7. Kristula M, Galligan D, Curtis C, Bartholomew R: Evaluation of a dairy herd improvement association test to predict chronic mastitis in dairy cows. *Prev Vet Med* 14:251-258, 1992.