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# Incidence and occurrence time of clinical mastitis in Holstein cows

**Ismaïl BOUJENANE[1,*], Jalila EL AIMANI[1], Khalid BY[2]**
[1]Department of Animal Production and Biotechnology, Institute of Agronomy and Veterinary Medicine, Rabat, Morocco
[2]Mazaria Farm, Larache, Morocco

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**Abstract:** The aim of this study was to assess the incidence of clinical mastitis (CM) and its risk factors in 1725 Holstein cows. Data were collected from a private farm from 2008 to 2012. The analysis of risk factors, performed with logistic regression, showed that cows at parity 2, 3, and 4 had 65%, 88%, and 115% risk of mastitis, respectively. This risk was higher ($P < 0.001$) than in cows at first parity. Cows that calved from October to January had the highest ($P < 0.05$) risk of mastitis. The number of mastitis cases in cows at lactation 2, 3, and 4 was 18.5%, 25.9%, and 40.7%, respectively, and was higher compared to cows at first lactation. Furthermore, cows that calved from July to September showed the highest number of mastitis cases. Gamma regression parameter estimates showed that the onset time of mastitis 1 and 2 in cows that calved from October to January occurred at 16.3 and 8.5 days, respectively. This was later than cows calving from July to September. The study concluded that it is necessary to examine cows for CM during the first 2 months of lactation in order to prevent mastitis and decrease its rate of incidence.

**Key words:** Cow, clinical mastitis, time of mastitis occurrence, risk factors

## 1. Introduction

In the last decade, the dairy sector in Morocco has known rapid development. However, this expansion has not been accompanied by optimal management techniques. Consequently, several diseases have appeared, including clinical mastitis (CM), one of the major diseases in Moroccan dairy farms. Mastitis is one of the most common and costly diseases in dairy cattle (1). Its incidence is approximately 30–40 cases per 100 cows per year (2). Mastitis reduces milk production and milk quality. Average economic losses in the United States due to mastitis amount to $200 per cow per year (3). Moreover, the loss of milk production in a clinical case has been found to vary from 0 to 902 kg or from 0% to 11% of the 305-day milk yield (4). Additionally, the risk of culling following a case of CM increases by a factor of 1.5–5 (5). Likewise, mastitis increases treatment costs, labor costs, veterinary fees, risk of culling, and risk of death. The economic impact of CM is approximately 33%–38% of the total health cost of dairy herds (6,7). In Sweden, the number of veterinary-treated cases of mastitis was 18.3 per 100 lactations in 2000–2001, and udder diseases were the second leading reason for culling in the year 2001, accounting for nearly 24% of culled cows (8). No studies of CM incidence have been conducted in Morocco. Therefore, an assessment of CM is necessary in order to obtain a clear impression about its incidence and risk factors.

The objective of this study was to assess the incidence of CM, the number of cases within a 305-day lactation period, and the time of occurrence in a private dairy herd, in order to decrease the rate of incidence.

## 2. Materials and methods

### 2.1. Herd management and data recording

Data were collected in a commercial dairy farm in northern Morocco. The mean annual temperature is 18.7 °C, varying from 0 °C in February to 41 °C in August. The mean annual rainfall is 600 mm, mainly occurring from November to March.

Data on Holstein cows were recorded between July 2008 and December 2012. Average age at calving was 47.6 months, ranging from 23 to 82 months, and average lactation number was 2.31, ranging from 1 to 4. During the study, the mean milk production per cow was 8066 kg per 305-day lactation.

All cows were housed in free-stall barns. They were observed for estrus twice daily, in the morning and afternoon. Cows with estrus were inseminated within 1 h of detection. All lactating cows were fed the same diet, which was formulated to meet the nutrient requirements

* Correspondence: i.boujenane@iav.ac.ma

of a lactating Holstein cow weighing 650 kg and producing 25–35 kg of milk daily.

The cows were milked in fully automated milking parlors equipped with automatic milking machines. Cows were milked 2–3 times daily, depending on their lactation stage. Teat dipping was routinely performed at milking. The milking equipment was evaluated routinely and maintained according to the recommendations of the manufacturer.

### 2.2. Definition of CM

Cows were examined for symptoms of CM by the milking personnel at each milking. The mastitis data considered were CM cases detected at milking by inflammation of the udder (heat, swelling, or discoloration), or by the presence of clots, flakes, and water in the milk from one or more quarters. Mastitis cases were systematically treated with antibiotics. We only considered CM cases separated by at least 15 days within the 305-day lactation period. For each case, we recorded the time of mastitis occurrence and the cow characteristics chosen to model the variables studied.

### 2.3. Statistical analysis

Mastitis was defined as a binary trait (0 = no mastitis, 1 = mastitis), based on whether a cow had at least one mastitis case between calving and 305 days postcalving. Thus, the data of the outcome were a sequence of zeros and ones for each cow. The initial data file included 1928 records. After editing, we excluded those cases that occurred during the dry period before calving; cows with mastitis that occurred more than 305 days after calving; cows with an unknown birth and calving date; cows without a lactation number; and cows with a lactation number that did not correspond to a minimum age at calving. The defined minimum and maximum ages for calvings 1, 2, 3, and 4 were determined after the analysis of the frequency distribution of the ages at different calving times. These ages were 21–38, 34–52, 46–68, and 58–84 months, respectively. Discarded records represented 10.5% of the initial data. Finally, 1725 records were analyzed.

Logistic binary regression was used to model the incidence of CM. The logistic model, fitted with CM as the outcome variable (present: 1, absent: 0), included fixed effects of the risk factors of parity (4 levels: 1, 2, 3, and 4 or greater), season of calving (3 levels: February–June, July–September, and October–January), and year of calving (5 levels: 2008, 2009, 2010, 2011, and 2012). Interactions were not included in the model because a biological interpretation would have been inconsequential. Parameter estimates, standard errors, odds ratios, and 95% confidence intervals were obtained from PROC Logistic (SAS Institute, USA). The odds ratio is a measure of the likelihood of an outcome occurring in observations with a given risk factor compared with observations without the risk factor. An odds ratio of 1.0 implies that observations with a risk factor are equally likely to have the same outcome as observations without the risk factor. Reference classes consisted of parity 1, the calving season of October–January, and the 2011 calving classes, which had the highest number of observations.

The number and occurrence time of mastitis cases during the 305-day lactation period were analyzed with the Poisson and gamma regression models, respectively, using PROC Genmod (9). The mastitis onset times were studied by considering the time of mastitis occurrences 1, 2, and 3 in cows with at least one incidence during the 305-day lactation period. The models used to analyze the number of mastitis cases and the time of their occurrence included the same risk factors as those used for the study of mastitis incidence (parity, calving season, and calving year). The significance of coefficients was assessed by Wald chi-square tests. Moreover, we computed the least square means of the number of mastitis cases and the time of mastitis occurrences that corresponded to the specified effects for the linear predictor part of the model.

## 3. Results

### 3.1. Incidence of CM

The average percentage of cows with at least one case of CM during the 305-day lactation period was 26.9%, since mastitis occurred in 464 of 1725 cows. Parameter estimates of the logistic binary regression and odds ratios for CM are reported in Table 1. The analysis of risk factors showed that parity, calving season, and calving year had significant effects on the incidence of CM ($P < 0.05$ to $P < 0.001$). The risk of CM increased with parity. Thus, cows at parity 2, 3, and 4 had a 65%, 88%, and 115% risk of mastitis, respectively. This was higher than cows at parity 1 (reference class). The results of the Wald chi-square test showed significant differences between these parities and the reference class ($P < 0.001$).

CM incidence was also influenced by calving season. The highest incidence was noted in the October–January calving period. Cows that calved from February to June and from July to September had a 24% and 30% risk of mastitis, respectively, lower than those that calved from October to January. Differences between the 2 previous seasons and the reference class were significant ($P < 0.05$).

The incidence of mastitis differed among calving years. It was low during 2008 and 2012 and high during 2009 and 2010. Thus, cows that calved in 2008 and 2012 had a 65% and 36% risk of mastitis, respectively. This was lower than in cows that calved in 2011. Cows that calved in 2009 and 2010 had a 30% and 71% risk of mastitis, respectively, higher than cows that calved in 2011. Differences between the reference class (2011) and the other calving years were significant ($P < 0.05$ to $P < 0.001$), except for the year 2009 ($P > 0.05$).

**Table 1.** Parameter estimates ± standard error (SE), P-value, odds ratio, and 95% Wald confidence interval (CI) from logistic binary regression model for incidence of clinical mastitis.

| Risk factors | Number | Parameter estimate ± SE | P-value | Odds ratio | 95% Wald CI | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Intercept | 1725 | −1.40 ± 0.14 | 0.0001 | | | |
| Parity | | *** | | | | |
| 1 | 507 | Reference | | 1.00 | | |
| 2 | 455 | 0.50 ± 0.16 | 0.0001 | 1.65 | 1.20 | 2.27 |
| 3 | 506 | 0.63 ± 0.15 | 0.0001 | 1.88 | 1.39 | 2.54 |
| 4 | 257 | 0.77 ± 0.19 | 0.0001 | 2.15 | 1.48 | 3.13 |
| Season of calving | | * | | | | |
| February–June | 575 | −0.28 ± 0.13 | 0.0324 | 0.76 | 0.59 | 0.98 |
| July–September | 301 | −0.35 ± 0.16 | 0.0283 | 0.70 | 0.51 | 0.96 |
| October–January | 849 | Reference | | 1.00 | | |
| Year of calving | | *** | | | | |
| 2008 | 62 | −1.05 ± 0.48 | 0.0302 | 0.35 | 0.14 | 0.90 |
| 2009 | 102 | 0.26 ± 0.24 | 0.2799 | 1.30 | 0.81 | 2.10 |
| 2010 | 446 | 0.53 ± 0.13 | 0.0001 | 1.71 | 1.31 | 2.22 |
| 2011 | 969 | Reference | | 1.00 | | |
| 2012 | 146 | −0.45 ± 0.22 | 0.0457 | 0.64 | 0.41 | 0.99 |

*: P < 0.05
***: P < 0.001

### 3.2. Number of mastitis cases per lactation

The number of CM cases during the 305-day lactation period varied from 1 to 6. The reoccurrence rate (total number of cases per number of lactation with at least one case) averaged 2.00, with a coefficient of variation of 49%. The frequency distribution showed that 33.2%, 44.0%, and 15.9% of cows had 1, 2, and 3 mastitis cases within the 305-day lactation period, whereas 7.12% had 4 to 6 mastitis cases (Figure 1).

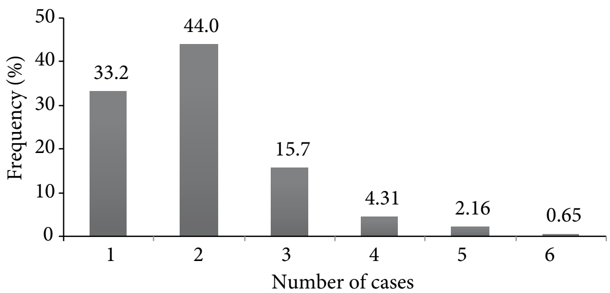


**Figure 1.** Reoccurrence rate of clinical mastitis within 305-day lactation period.

Parameter estimates of Poisson regression and least square means for the number of CM cases during 305-day lactation are reported in Table 2. Parity had a significant effect on the number of mastitis cases (P < 0.001). Least square means, corresponding to the parity for the linear predictor part of the model, increased between parity 1 and 4. The number of mastitis cases at parity 2, 3, and 4 was 18.5%, 25.9%, and 40.7%, respectively, higher than parity 1. The results of the Wald chi-square test showed that differences between parities 1 and 2 on the one hand and parity 4 on the other hand were significant (P < 0.05 and P < 0.001), whereas differences between parities 3 and 4 were not significant (P > 0.05).

Calving season had a significant effect on the number of mastitis cases (P < 0.05). Cows that calved from July to September showed a number of mastitis cases ranging from 0.20 and 0.51, respectively, higher than cows that calved from February to June and October to January (P < 0.01 and P < 0.001, respectively).

The number of CM cases that occurred during the 305-day lactation period was influenced by calving year (P <

**Table 2.** Parameter estimates ± standard error (SE), 95% confidence interval (CI), P-values, and least square mean (LSM) ± SE from Poisson regression model for the number of mastitis cases during 305-day lactation.

| Factors | Number | Parameter estimate ± SE | 95% Wald CI Lower | Upper | P-value | LSM ± SE |
|---|---|---|---|---|---|---|
| Intercept | 464 | 0.75 ± 0.09 | 0.56 | 0.93 | 0.0001 | 2.28 ± 0.12 |
| Parity | | *** | | | | |
| 1 | 104 | −0.34 ± 0.07 | −0.48 | −0.19 | 0.0001 | 1.89 ± 0.13 |
| 2 | 126 | −0.17 ± 0.07 | −0.30 | −0.03 | 0.0144 | 2.24 ± 0.13 |
| 3 | 157 | −0.11 ± 0.06 | −0.23 | 0.02 | 0.0908 | 2.38 ± 0.15 |
| 4 | 77 | Reference | | | | 2.66 ± 0.19 |
| Season of calving | | *** | | | | |
| February–June | 142 | 0.14 ± 0.05 | 0.05 | 0.24 | 0.0032 | 2.32 ± 0.14 |
| July–September | 71 | 0.22 ± 0.06 | 0.11 | 0.34 | 0.0002 | 2.52 ± 0.18 |
| October–January | 251 | Reference | | | | 2.01 ± 0.10 |
| Year of calving | | *** | | | | |
| 2008 | 5 | 0.18 ± 0.22 | −0.26 | 0.62 | 0.4233 | 2.45 ± 0.51 |
| 2009 | 32 | 0.37 ± 0.11 | 0.15 | 0.59 | 0.0010 | 2.96 ± 0.23 |
| 2010 | 154 | 0.03 ± 0.09 | −0.15 | 0.21 | 0.7496 | 2.11 ± 0.09 |
| 2011 | 243 | −0.05 ± 0.09 | −0.22 | 0.13 | 0.5806 | 1.95 ± 0.06 |
| 2012 | 30 | Reference | | | | 2.05 ± 0.18 |

***: P < 0.001

0.001). The lowest number of cases was recorded for 2011. The number of mastitis cases for 2008, 2009, 2010, and 2012 was 25.6%, 51.8%, 8.20%, and 5.13%, respectively, higher than the number of cases recorded for 2011 (Table 2).

### 3.3. Time of CM occurrence

The time of mastitis occurrences 1, 2, and 3 in cows with at least one CM case during the 305-day lactation period averaged 69.1, 139.5, and 187.8 days postcalving, respectively (Table 3). Intervals between 1st and 2nd and between 2nd and 3rd mastitis cases were 70.4 and 48.3 days, respectively. The time of mastitis occurrence for cows affected once during the 305-day lactation period was 95.8 days postcalving. However, for cows affected twice during the same period, the 1st mastitis case occurred at 64.3 days postcalving and the 2nd at 158.1 days, with an interval of 93.8 days. The onset times of mastitis 1, 2, and 3 for cows that showed 3 mastitis incidences during the 305-day lactation period averaged 43.1, 112.5, and 202.0 days postcalving, respectively. The interval between mastitis cases was 69.4 days for cases 1 and 2, and 89.5 days for cases 3 and 4.

Frequency distribution of mastitis occurrence is displayed in Figure 2. Although mastitis can occur any time during lactation, the modal class for mastitis 1 was 3 weeks postcalving, for mastitis 2 was 12–15 weeks postcalving, and for mastitis 3 was 24–27 weeks postcalving.

Least square means for mastitis occurrences 1, 2, and 3 in cows with at least one mastitis case during the 305-day lactation period was 71.4, 147.1, and 197.0 days postcalving, respectively. The onset time of mastitis 1 and 2 was influenced by calving season (P < 0.05), although not by parity or calving year (P > 0.05). The onset time of mastitis 3 was not affected by any of the risk factors studied (Table 4). The onset time of mastitis 1 and 2 was precocious for cows that calved from October to January (61.0 and 135.9 days, respectively). However, it occurred 16.3 and 8.5 days later than in cows that calved from July to September, respectively. Differences between cows that calved between February and June and those that calved from October to January were significant (P < 0.05), whereas differences between the latter group and cows that calved from July to September were not significant (P > 0.05).

## 4. Discussion

The percentage of cows with at least one CM case during the 305-day lactation period averaged 26.9. This incidence rate of mastitis is slightly lower than the 32.2% reported by Lescourret et al. (10). Nonetheless, it is higher than the estimated 23% (11). The higher incidence rate of mastitis found in the present study can be explained by the overlap between calving period and wet season, and also by the

**Table 3.** Descriptive statistics of mastitis occurrence times during the 305-day lactation period.

| Variable[1] | Number | Arithmetic mean | Standard deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| Number of cases | 464 | 2.00 | 0.98 | 1 | 6 |
| IC-M1 (days) | 464 | 69.1 | 61.4 | 1 | 293 |
| IC-M2 (days) | 309 | 139.5 | 73.4 | 18 | 301 |
| IC-M3 (days) | 108 | 187.8 | 64.3 | 59 | 305 |
| IC-M1/1 (days) | 154 | 95.8 | 72.1 | 2 | 293 |
| IC-M1/2 (days) | 204 | 64.3 | 54.2 | 1 | 279 |
| IC-M2/2 (days) | 203 | 158.1 | 74.4 | 18 | 301 |
| IC-M1/3 (days) | 73 | 43.1 | 39.3 | 2 | 166 |
| IC-M2/3 (days) | 73 | 112.5 | 59.2 | 18 | 273 |
| IC-M3/3 (days) | 73 | 202.0 | 62.0 | 88 | 305 |

[1]IC-M1: Time of first mastitis onset for cows with at least one mastitis case
IC-M2: Time of second mastitis onset for cows with at least one mastitis case
IC-M3: Time of third mastitis onset for cows with at least one mastitis case
IC-M1/1: Time of first mastitis onset for cows with one mastitis case
IC-M1/2: Time of first mastitis onset for cows with 2 mastitis cases
IC-M2/2: Time of second mastitis onset for cows with 2 mastitis cases
IC-M1/3: Time of first mastitis onset for cows with 3 mastitis cases
IC-M2/3: Time of second mastitis onset for cows with 3 mastitis cases
IC-M3/3: Time of third mastitis onset for cows with 3 mastitis cases

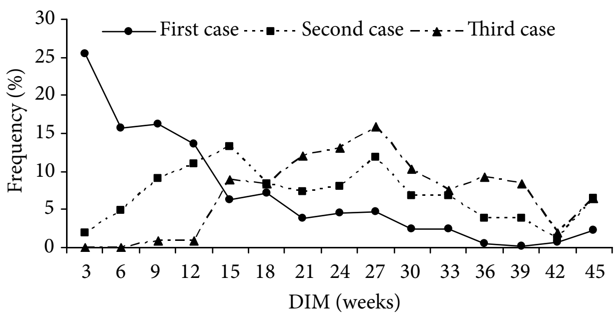


**Figure 2.** Distribution of the time of the first 3 clinical mastitis occurrences within 305-day lactation period. DIM = Days in milk.

over-reporting of mastitis cases due to the accurate health recording practiced on the farm.

The increased risk of CM in parity is consistent with the results of several authors (8,12–15). Steeneveld et al. (16) reported that the incidence rate of CM in the first 10 days of lactation was higher in heifers than in multiparous cows. However, after 10 days of lactation, it was higher in multiparous cows than in heifers. Koeck et al. (1) reported that the overall CM frequency — the percentage of cows with at least one case of CM in the period from 10 days prior to 241 days after first calving — was 10.1%. The increase in mastitis incidence with parity may be explained by physical changes in the udder and the permeability of teats' sphincter to pathogen agents.

CM incidence was also influenced by calving season. Lescourret et al. (10), Breen et al. (15), and Steeneveld et al. (16) reported that calving month played an important role, and that the incidence of mastitis was greater when calving took place in early autumn or winter. Likewise, Gröhn et al. (17) found that cows calving between December and February or between June and August had the highest risk of mastitis. However, Carlén et al. (8) did not observe a clear effect of calving month and year (January 1995–December 2000) on mastitis frequency. The eminent risk of mastitis during the October–January calving (wet season) may be explained by the free and open housing used in the farm under study, which increased infectious agents in the cow bedding.

The number of CM cases during the 305-day lactation period varied from 1 to 6. The frequency distribution showed that 33.2%, 44.0%, and 15.9% of cows had 1, 2, and 3 mastitis cases during the 305-day lactation period. Lescourret et al. (10) reported that cows with 1, 2, 3, and 4 mastitis cases in lactation represented 68.5%, 24.6%, 4.45%, and 2.3%, whereas Carlén et al. (18) found that less than 1% of the cows had more than one case (maximum 4) of CM.

The significant effect of parity on the number of CM cases in the current study is in agreement with the result of Morse et al. (19). However, it is not consistent with the result of Lescourret et al. (10), who did not find any significant effect of parity on the number of CM cases

**Table 4.** Parameter estimates ± standard error (SE), P-value, and least square mean (LSM) ± SE from gamma regression model for onset time of mastitis 1, 2, and 3 in cows with at least one mastitis case during the 305-day lactation period.

| | Time of mastitis onset 1 | | | | Time of mastitis onset 2 | | | | Time of mastitis onset 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factors | Number | Parameter estimate ± SE | P-value | LSM ± SE (days) | Number | Parameter estimate ± SE | P-value | LSM ± SE (days) | Number | Parameter estimate ± SE | P-value | LSM ± SE (days) |
| Intercept | 464 | 3.73 ± 0.20 | 0.0001 | 71.4 ± 7.76 | 309 | 4.66 ± 0.13 | 0.0001 | 147.1 ± 11.5 | 108 | 5.33 ± 0.16 | 0.0001 | 197.0 ± 14.5 |
| Parity | | NS | | | | NS | | | | NS | | |
| 1 | 104 | 0.18 ± 0.15 | 0.2481 | 77.0 ± 11.0 | 52 | 0.21 ± 0.11 | 0.0620 | 161.5 ± 17.5 | 10 | −0.14 ± 0.13 | 0.2890 | 175.4 ± 22.3 |
| 2 | 126 | 0.11 ± 0.15 | 0.4631 | 71.9 ± 8.75 | 86 | 0.12 ± 0.10 | 0.2541 | 147.3 ± 12.8 | 38 | 0.11 ± 0.10 | 0.2931 | 224.2 ± 16.4 |
| 3 | 157 | 0.12 ± 0.14 | 0.3920 | 72.6 ± 9.32 | 113 | 0.14 ± 0.09 | 0.1450 | 150.2 ± 13.8 | 35 | −0.06 ± 0.09 | 0.5274 | 190.0 ± 18.0 |
| 4 | 77 | Reference | | 64.5 ± 9.95 | 58 | Reference | | 131.0 ± 14.1 | 25 | Reference | | 201.6 ± 19.8 |
| Season of calving | | * | | | | * | | | | NS | | |
| February–June | 142 | 0.23 ± 0.10 | 0.0216 | 77.3 ± 9.82 | 104 | 0.18 ± 0.07 | 0.0149 | 162.0 ± 14.6 | 36 | 0.15 ± 0.08 | 0.0469 | 217.0 ± 19.5 |
| July–September | 71 | 0.23 ± 0.13 | 0.0755 | 77.1 ± 11.9 | 54 | 0.06 ± 0.09 | 0.5073 | 144.4 ± 15.5 | 22 | 0.02 ± 0.09 | 0.8452 | 189.4 ± 19.8 |
| October–January | 251 | Reference | | 61.0 ± 6.53 | 151 | Reference | | 135.9 ± 10.5 | 50 | Reference | | 186.0 ± 13.0 |
| Year of calving | | NS | | | | NS | | | | NS | | |
| 2008 | 5 | 0.87 ± 0.47 | 0.0637 | 128.2 ± 55.6 | 3 | 0.49 ± 0.34 | 0.1516 | 210.5 ± 68.2 | 2 | 0.14 ± 0.29 | 0.6163 | 247.1 ± 62.1 |
| 2009 | 32 | −0.08 ± 0.25 | 0.7458 | 49.6 ± 9.15 | 30 | −0.003 ± 0.16 | 0.9867 | 128.4 ± 14.7 | 15 | −0.21 ± 0.18 | 0.2501 | 173.5 ± 18.1 |
| 2010 | 154 | 0.31 ± 0.20 | 0.1165 | 73.4 ± 6.39 | 100 | 0.06 ± 0.13 | 0.6344 | 137.1 ± 8.30 | 35 | −0.18 ± 0.17 | 0.2932 | 178.7 ± 11.2 |
| 2011 | 243 | 0.32 ± 0.19 | 0.0915 | 73.9 ± 4.78 | 152 | 0.11 ± 0.12 | 0.3660 | 144.2 ± 6.93 | 51 | −0.17 ± 0.17 | 0.3206 | 181.1 ± 9.45 |
| 2012 | 30 | Reference | | 53.8 ± 9.79 | 24 | Reference | | 128.7 ± 15.4 | 5 | Reference | | 214.0 ± 34.9 |

NS: P > 0.05
*: P < 0.05

per lactation. Nevertheless, mastitis incidence tended to increase with parity, although not significantly. Such discrepancies may have partly resulted from differences in culling policies with regard to mastitis, which may bias the assessment of the parity effect.

Calving season had a significant effect on the number of mastitis cases ($P < 0.05$). Cows affected in summer had a higher risk of mastitis during the 305-day lactation period than cows that calved during the other seasons. Moreover, the increase in number of mastitis cases in summer (July–September) may be explained by the proliferation of bacteria due to hot temperatures in the surrounding environment, mainly near watering troughs.

The number of CM cases was also influenced by year of calving ($P < 0.001$). Differences among years may be explained by the amount of annual rainfall, which may affect the housing and bedding conditions of cows and hence the number of mastitis cases.

For cows with at least one incidence of CM during the 305-day lactation period, mastitis occurrences 1, 2, and 3 averaged 69.1, 139.5, and 187.8 days postcalving, respectively. The onset time of mastitis 1, 2, and 3 for cows that had 3 mastitis cases during lactation averaged 43.1, 112.5, and 202.0 days postcalving, respectively. Thus, as the first mastitis occurs earlier, the number of mastitis cases during the 305-day lactation is higher.

Frequency distribution of mastitis occurrence showed that although mastitis can occur at any time during lactation, the modal class for mastitis 1 was approximately 3 weeks postcalving, for mastitis 2 from 12 to 15 weeks postcalving, and for mastitis 3 from 24 to 27 weeks postcalving. In the current study, the onset time for mastitis 1 is in agreement with the results of several authors (1,8,10). The high frequency of cows affected by mastitis at the beginning of lactation may be explained by their high susceptibility due to the increased physiological demands after calving, and possibly to the pronounced sensitivity of the udder to infectious agents. It may also be attributed to anterior infections caused by bacteria during the dry period or to new infections.

In conclusion, the results of this study showed that mastitis incidence was high in a well-managed herd with good recording. Moreover, cows may show several mastitis cases during the 305-day lactation period. Most cows were affected within 2 months after calving. Risk factors associated with CM (parity, calving season, and calving year) were found to be significant. We conclude that it is necessary to examine cows for CM during the first 2 months of lactation in order to prevent mastitis and decrease the rate of its incidence.

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## References

1. Koeck A, Miglior F, Kelton DF, Schenkel FS. Alternative somatic cell count traits to improve mastitis resistance in Canadian Holsteins. J Dairy Sci 2012; 95: 432–439.
2. Heringstad B, Klemetsdal G, Ruane J. Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. Livest Prod Sci 2000; 64: 95–106.
3. Smith KL, Hogan JS. The world of mastitis. In: Proceedings of the 2nd International Symposium on Mastitis and Milk Quality. Vancouver, Canada: NMC; 2001. pp. 1–12.
4. Hagnestam C, Emanuelson U, Berglund B. Yield losses associated with clinical mastitis occurring in different weeks of lactation. J Dairy Sci 2007; 90: 2260–2270.
5. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis economics in dairy cattle herds. Vet Res 2003; 34: 475–491.
6. Kossaibati MA, Esslemont RJ. The costs of production diseases in dairy herds in England. Vet J 1997; 154: 41–51.
7. Fourichon C, Seegers H, Beaudeau F, Verfaille L, Bareille N. Health-control costs in dairy farming systems in western France. Livest Prod Sci 2001; 68: 141–156.
8. Carlén E, Strandberg E, Roth A. Genetic parameters for clinical mastitis, somatic cell score, and production in the first three lactations of Swedish Holstein cows. J Dairy Sci 2004; 87: 3062–3070.
9. SAS. SAS/STAT User's Guide (Release 9.0). Cary, NC, USA: SAS Institute Inc.; 2002.
10. Lescourret F, Coulon JB, Faye B. Predictive model of mastitis occurrence in the dairy cow. J Dairy Sci 1995; 78: 2167–2177.
11. Olde Riekerink RGM, Barkema HW, Kelton DF, Scholl DT. Incidence rate of clinical mastitis on Canadian dairy farms. J Dairy Sci 2008; 91: 1366–1377.
12. Pösö J, Mäntysaari EA. Relationships between clinical mastitis, somatic cell score, and production for the first three lactations of Finnish Ayrshire. J Dairy Sci 1996; 79: 1284–1291.
13. Nielsen US, Pedersen GA, Pedersen J, Jensen J. Genetic correlations among health traits in different lactations. In: Proceedings of the International Workshop on Genetic Improvement of Functional Traits in Cattle; Health. Uppsala, Sweden: Interbull; 1997. pp. 68–77.
14. Heikkilä AM, Nousiainen JI, Pyörälä S. Costs of clinical mastitis with special reference to premature culling. J Dairy Sci 2012; 95: 139–150.

15. Breen JE, Green MJ, Bradley AJ. Quarter and cow risk factors associated with the occurrence of clinical mastitis in dairy cows in the United Kingdom. J Dairy Sci 2009; 92: 2551–2561.

16. Steeneveld W, Hogeveen H, Barkema HW, van den Broek Huirne RBM. The influence of cow factors on the incidence of clinical mastitis in dairy cows. J Dairy Sci 2008; 91: 1391–1402.

17. Gröhn YT, Eicker SW, Hertl JA. The association between previous 305-day milk yield and disease in New York state dairy cows. J Dairy Sci 1995; 78: 1693–1702.

18. Carlén E, Grandinson K, Emanuelson U, Strandberg E. Random regression models for genetic evaluation of clinical mastitis in dairy cattle. Animal 2009; 3: 1100–1108.

19. Morse D, DeLorenzo MA, Wilcox CJ, Natzke RP, Bray DR. Occurrence and reoccurrence of clinical mastitis. J Dairy Sci 1987; 70: 2168–2175.