

## THE INFLUENCE OF LATERALITY ON MASTITIS INCIDENCE IN DAIRY CATTLE – PRELIMINARY STUDY

B. H. KIKKERS<sup>1</sup>, L. ÓZSVÁRI<sup>2</sup>, F. J. C. M. VAN EERDENBURG<sup>1</sup>, Á. CS. BAJCSY<sup>3</sup>  
and O. SZENCI<sup>3\*</sup>

<sup>1</sup>Department of Farm Animal Health, Faculty of Veterinary Medicine, University of Utrecht, Yalelaan 7, 3584 CL Utrecht, The Netherlands; <sup>2</sup>Department of State Veterinary Medicine and Agricultural Economics, Faculty of Veterinary Science, Szent István University, Budapest, Hungary; <sup>3</sup>Clinic for Large Animals, Faculty of Veterinary Science, Szent István University, H-2225 Üllő, Dóra major, Hungary

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During summer (June to August) of continental weather conditions treated mastitis episodes at a large Hungarian dairy farm were studied to determine the relationship between the distribution of mastitis among quarters of the udder and the lying behaviour (laterality) of the cows. There was a significant difference in laterality between cows that developed mastitis and cows that did not. Cows that developed mastitis, had an increased left laterality in lying, which was related to the increased probability of a right-sided mastitis episode. However, the effect was not significant due to the large variation in the data. At the same time, significantly more cows with clinical mastitis had a somatic cell count (SCC) higher than that of cows without clinical mastitis. While a significant correlation was found between the occurrence of treated mastitis cases and SCC > 1,000,000, no statistically significant differences could be observed in milk production between the two groups.

**Key words:** Dairy cattle, laterality, mastitis, incidence, SCC

Research on the distribution of clinical mastitis revealed that the incidence of clinical mastitis on udder-quarter level often differs from a random distribution (Flock and Zeidler, 1969; Grootenhuis, 1975; Adkinson et al., 1993; Barkema et al., 1997). Several authors found the incidence of clinical mastitis to be higher in rear quarters than in front quarters (Rendel and Sundberg, 1962; Flock and Zeidler, 1969; Grootenhuis, 1975; Batra et al., 1977; Adkinson et al.,

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\*Corresponding author: Ottó Szenci; E-mail: Szenci.Otto@aotk.szie.hu;  
Fax: 0036 (29) 521-303

1993; Miltenburg et al., 1996; Barkema et al., 1997). Deviation from expectation of observed mastitis incidence on quarter level could be attributed to differences in risk factors with bacterial infections around the teat opening between the quarters of the bovine udder in developing clinical mastitis (Barkema et al., 1997). One of the factors that might influence the incidence of clinical mastitis on quarter level is the lying behaviour of the animal involved.

Cows tend to increase the habit of lying on their right side (right laterality) with increasing age. At the same time, cows with increasing gestation length tend to have an increasing left laterality (Arave and Walters, 1980). Nottbohm (1928) suggested that, as a result of left laterality, mastitis most often occurs in the left-hind quarters. Ewbank (1966) found that the greater the proportion of total lying time a cow spent on one particular side, the greater the difference in subclinical mastitis status between the sides, with the highest status for the relatively most lain-on half of the udder. In the lying cow, the underlying udder-half may be affected by the cold floor and be predisposed to infection by environmental pathogens. Also an injury of the underlying udder-half by tramping could more likely be followed by infections. An uneven distribution in laterality of cows might impose a different risk factor for the quarters of the udder in developing clinical mastitis caused by environmental pathogens. Exposure to environmental pathogens occurs between milkings [with the highest probability within 30 min (max. 60) after milkings] and is not limited to the milking process, which is in contrast to contagious pathogens (Eberhart and Buckalew, 1972, 1977; Bramley et al., 1981; Eberhart et al., 1983).

Therefore, the objective of the present study was to investigate the role of the laterality of cows on the incidence of the side of the udder at which clinical (treated) mastitis occurs.

## Materials and methods

### *Animals and housing*

This study was conducted at a Hungarian dairy farm with an average herd size of 950 Holstein-Friesian cows. The cows were individually labelled for identification with continuous numbering. The lactating herd was divided into six production groups, each of which was housed in free-stall loose housing system. The cubicles, serving for the resting period of the individual cows, had a sand floor and a layer of straw was provided as bedding. During the afternoon and at night the cows had access to a paddock outside the shed.

The animals were fed three times a day in a feed bunk along the open side of the shed. The ration consisted of a mixture of maize silage, green alfalfa and soybean completed with concentrate according to the production level of the group. Alfalfa hay and water were available *ad libitum*. All rations were fed to meet the NRC recommendations for essential nutrients (National Research

Council, 1989). Milking took place twice daily, starting at 5:00 a.m. and 4:30 p.m. The groups were milked separately using two double-sided herringbone milking parlours with 24 stands each (Alfa Laval, Lund, Sweden). Iodine-containing, post-milking teat dipping solutions were routinely applied. Feed was provided in the feed bunks along the open side of the shed after milking to prevent the lying down of the cows with open teats after milking, and thereby to avoid contamination of the udder with environmental pathogens.

The mean age of the cows was 4.7 years. The mean lactation level was 8,720 kg milk with 3.1% of fat. The mean lactation length was 349 days. The general hygienic condition of the herd was as usual. The somatic cell count (SCC) of the bulk tank milk varied from 373,000 to 569,000 in the year of the experiment, according to the results of the milk tests that had been performed by the dairy industry every month. Disinfectants were routinely used to prevent contamination by environmental pathogens in the calving shed.

#### *Mastitis recording*

Detailed records on treated mastitis were kept in the farm's log. These records included the identification of cows, the distribution among quarters, the date of treatment and the type of antibiotics used. A treated case of mastitis was defined as any intramammary antibiotic treatment. A separate case was defined in this study as one in which at least 14 days had elapsed since any previous treatment in the same quarter.

According to the farm technology, milk bacteriologic culturing and determination of antibiotic resistance were performed continuously, however, only in selected cases. Based on these data, *Staphylococcus aureus* was the main bacterial pathogen of mastitis episodes. This bacterium alone was responsible for 40 to 50% of all mastitis cases in the year of the study. Far less importance (5–10%) was attributed to other contagious mastitis pathogens (*Streptococcus agalactiae*, *Nocardia* spp.), while environmental pathogens (*E. coli*, *Klebsiella* spp., *Streptococcus uberis*) were detected in about 40 to 55% of the mastitis cases. Occasionally, contagious/environmental bacteria, such as *Streptococcus dysgalactiae*, were found. The mastitis episodes were treated with antibiotics based on the results of the resistance tests.

Lactation periods between June and October were analysed in this study. The temperature and the humidity corresponded to the continental weather conditions, as usual. The SCC and milk production (kg) of cows included in the experiment were analysed on a monthly basis from June to October. The SCC and milk production indices were divided into two groups, based on the existence or lack of a detected mastitis. The values of the different groups were compared by analysis of variance. Furthermore, the monthly SCC indices were grouped according to their SCC values into three categories (< 400,000; 400,000–1,000,000; > 1,000,000 SCC/ml milk). The occurrence of mastitis in different

SCC categories and the monthly and overall occurrence of mastitis in each SCC category were compared by Chi<sup>2</sup>-tests.

#### *Analyses of laterality recordings and subsequent mastitis cases*

Two sheds, containing 227 fresh and high-producing (> 30 kg) dairy cows, were used for the laterality recordings. The lying position of the cows was recorded twice in the morning (1st recording: after the first feeding, 2nd recording: 1 h later) and twice again in the afternoon (1st recording: after the second feeding, 2nd recording: 1 h later), at moments when only a few cows were standing. Laterality recordings were performed during 15 consecutive days (from 20 June to 4 July). The herd was kept in a quiet disposition and they remained undisturbed when strangers entered the cowshed. Animals that were seen rising were not recorded.

The left-laterality fraction of each individual cow was used for analyses. This fraction was calculated by dividing the number of left laterality observations by the total number of observations on each cow. For the analysis of laterality recordings and subsequent mastitis cases, only those treated mastitis cases were used, which occurred within 8 weeks after the laterality recordings (from 5 July to 28 August). This criterion was applied to avoid bias due to changes in laterality during lactation. Cows, which developed mastitis episodes during the period of laterality recordings, were excluded from the analysis.

The relation between laterality and the side at which a subsequent mastitis episode occurred was analysed using logistic regression. Differences in left laterality fractions between cows that developed treated mastitis and cows that did not, were analysed using the Wald-test (S-Plus 2000) (Armitage and Berry, 1994).

### **Results**

After the laterality recordings had been performed, 48 of the 227 cows (21%) developed a mastitis episode within 8 weeks, which was then treated with antibiotics. The distribution of lactation stages in cows with treated mastitis were as follows: before Day 35: n = 1; between Days 35–100: n = 20; beyond Day 100: n = 27. According to the number of lactations: 1st lactation: n = 8; 2nd lactation: n = 10; 3rd lactation: n = 5; 4th lactation: n = 13; 5–7th lactation: n = 12. During the period of laterality recordings, three cows developed mastitis and were excluded from the analysis. The number of laterality observations, the left laterality fraction and the side at which the mastitis episode occurred are shown in Table 1.

Cows that developed treated mastitis on the left side (n = 27) had an average left laterality fraction of 0.556 ( $s^2 = 0.051$ ). Average left laterality fraction for cows developing treated mastitis on the right side (n = 21) was 0.613 ( $s^2 = 0.033$ ). Logistic regression showed an odds ratio on left-sided treated mastitis of 0.257 with increasing left laterality fraction ( $P = 0.65$ ).

**Table 1**

Number of observations, left laterality fraction and treated mastitis side

| Cow nr. | No. of observations | Left laterality fraction | Mastitis side | Cow nr. | No. of observations | Left laterality fraction | Mastitis side |
|---------|---------------------|--------------------------|---------------|---------|---------------------|--------------------------|---------------|
| 1       | 39                  | 0.462                    | Left          | 25      | 18                  | 0.722                    | Left          |
| 2       | 4                   | 0.250                    | Left          | 26      | 8                   | 0.375                    | Left          |
| 3       | 15                  | 0.333                    | Right         | 27      | 11                  | 0.455                    | Right         |
| 4       | 13                  | 0.615                    | Right         | 28      | 16                  | 0.500                    | Right         |
| 5       | 8                   | 1.000                    | Right         | 29      | 16                  | 0.500                    | Right         |
| 6       | 14                  | 0.429                    | Left          | 30      | 9                   | 0.444                    | Left          |
| 7       | 16                  | 0.625                    | Right         | 31      | 8                   | 1.000                    | Left          |
| 8       | 18                  | 0.444                    | Left          | 32      | 13                  | 0.692                    | Left          |
| 9       | 13                  | 1.000                    | Left          | 33      | 17                  | 0.824                    | Right         |
| 10      | 33                  | 0.515                    | Left          | 34      | 20                  | 0.800                    | Left          |
| 11      | 13                  | 0.385                    | Left          | 35      | 14                  | 0.714                    | Right         |
| 12      | 16                  | 0.625                    | Right         | 36      | 7                   | 0.714                    | Right         |
| 13      | 21                  | 0.286                    | Left          | 37      | 14                  | 0.786                    | Left          |
| 14      | 22                  | 0.364                    | Right         | 38      | 15                  | 0.800                    | Left          |
| 15      | 13                  | 0.692                    | Right         | 39      | 11                  | 0.364                    | Left          |
| 16      | 30                  | 0.567                    | Right         | 40      | 16                  | 0.500                    | Left          |
| 17      | 6                   | 0.833                    | Left          | 41      | 17                  | 0.765                    | Left          |
| 18      | 13                  | 0.385                    | Right         | 42      | 17                  | 0.647                    | Left          |
| 19      | 14                  | 0.214                    | Left          | 43      | 13                  | 1.000                    | Right         |
| 20      | 13                  | 0.692                    | Right         | 44      | 12                  | 0.500                    | Left          |
| 21      | 19                  | 0.421                    | Right         | 45      | 11                  | 0.545                    | Right         |
| 22      | 3                   | 0.667                    | Right         | 46      | 15                  | 0.600                    | Left          |
| 23      | 18                  | 0.500                    | Left          | 47      | 14                  | 0.643                    | Right         |
| 24      | 12                  | 0.167                    | Left          | 48      | 13                  | 0.538                    | Left          |

Cows that developed treated mastitis ( $n = 48$ ) had an average left laterality fraction of 0.581 ( $s^2 = 0.044$ ). Cows that did not develop treated mastitis ( $n = 176$ ) had an average left laterality fraction of 0.505 ( $s^2 = 0.044$ ). The Wald-test revealed that the differences in left laterality fractions between cows that developed treated mastitis and those that did not, were statistically significant ( $P = 0.026$ ).

The analysis of variance showed a significant difference ( $P < 0.001$ ) between the SCC values of cows with treated mastitis and those without mastitis at the following observations: 5 June, 4 September and 2 October (Fig. 1). No significant differences were found in the milk production in any of the two groups throughout the period studied (Fig. 2). The percentages of cows with and without mastitis, as grouped according to their different SCC values, based on monthly examinations, are presented in Fig. 3. The  $\chi^2$ -test showed significant differences in the category  $> 1,000,000$  SCC ( $P < 0.05$ ), but in the category  $< 400,000$  SCC and in that with  $400,000$ – $1,000,000$  SCC a non-significant difference could be found

between the occurrence of treated mastitis cases and the SCC values in the monthly evaluation. Besides, the result of Chi<sup>2</sup>-test showed that there was a significant difference between the total number of cows with treated mastitis and the SCC values by SCC categories ( $P < 0.001$ ). On a monthly basis the difference between the occurrence of treated mastitis cows and the SCC values by SCC categories was found to be significant in June ( $P < 0.01$ ), September and October (both with  $P < 0.001$ ), while no significant differences were found in July and August.

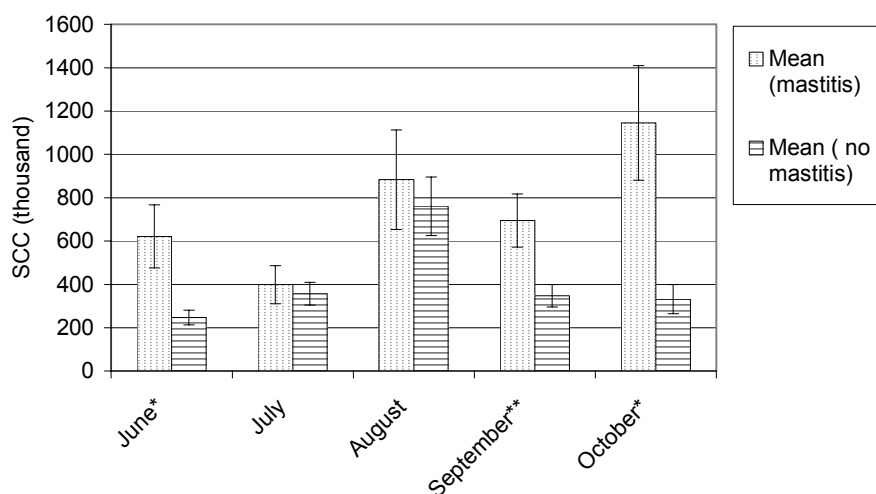


Fig. 1. The SCC values (mean, SE) of cows with mastitis and with no mastitis based on monthly examinations. \* $P < 0.001$ ; \*\* $P < 0.01$

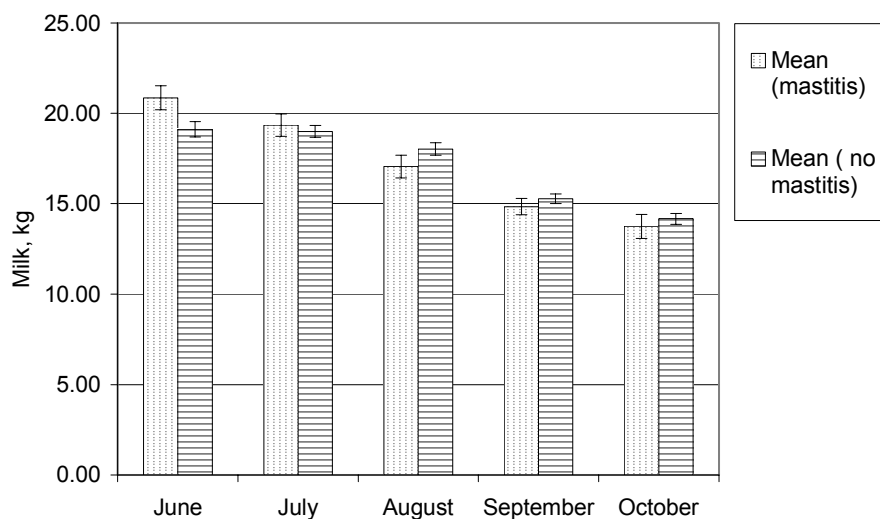


Fig. 2. The milk production (mean, SE) of cows with mastitis and with no mastitis based on monthly examinations

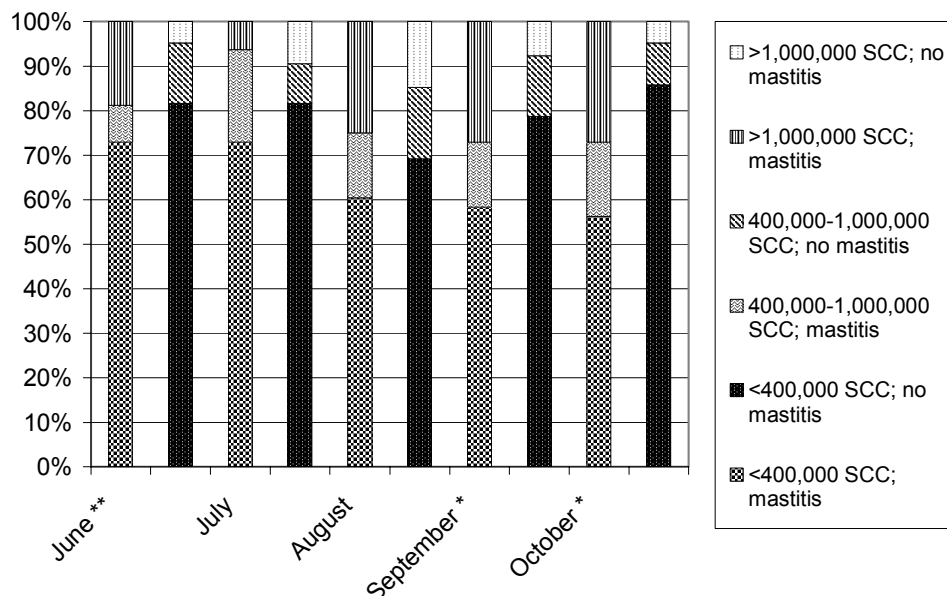


Fig. 3. The percentage of cows with mastitis and with no mastitis, grouped by different SCC values, based on monthly examinations. \* $P < 0.001$ ; \*\* $P < 0.01$

## Discussion

Although several studies did not show different incidences of clinical mastitis episodes between left and right quarters (Batra et al., 1977; Adkinson et al., 1993), Walsh (1985) reported a different incidence of clinical mastitis between left and right quarters in a herd with a one-sided milking parlour. The higher frequency of right-quarter mastitis episodes was caused by teat impaction. A higher prevalence of *Staphylococcus aureus* (*Staph. aureus*) infection in right quarters was found by Zadoks et al. (2001), but the transmission of *Staph. aureus* via teat cup liners (O'Shea, 1987) might also explain a higher incidence rate in the affected quarters, e.g. the right quarters. According to Barkema et al. (1997), intramammary infections also occurred more often in the right front quarters than in the left front quarters. They suggested that predisposing conditions that are disproportionate between left and right quarters could explain this uneven distribution. One of these conditions could be based on the laterality in lying.

We previously reported that the right quarters with treated mastitis were most frequent in all lactation stage groups and all age groups in this large-scale Hungarian dairy farm (Kikkers et al., 2004). According to the findings of Arave and Walters (1980), a shift toward left clinical quarters relative to right clinical quarters in subsequent lactation stage groups, and a shift toward right clinical quarters relative to left clinical quarters in subsequent age groups could be ex-

pected. However, the difference between observed frequencies of left or right treated quarters and the expected frequencies was not significant (Kikkers et al., 2004). A possible explanation could be that too few observations on mastitis episodes caused by environmental pathogens were taken to manifest the influence of laterality. Also an overestimation of the relation between laterality and the side on which mastitis occurs, could explain the result.

In this study, logistic regression revealed that in cows that developed mastitis, an increasing left laterality fraction was related to an increased probability of a right-sided mastitis episode. The 48 mastitis episodes and the related laterality fractions used in the logistic regression model contained a large variation that could not be explained by the model. Although the result was not significant, it is in contrast to the expected results. Several authors suggested that, in the lying cow, the underlying udder-half might be predisposed to infection due to increased exposure to environmental bacteria (Nottbohm, 1928; Bryan and Taylor, 1938; Ewbank, 1966). The present results imply an increased probability of the upper-half of the udder in a lying cow to be affected when the cow develops mastitis. To explain this result one has to realise that the layer of straw bedding insulates the floor, preventing the underlying udder-half from cooling down. In contrast, the overlying udder-half could be even more exposed to cold due to the wind in an open barn. As has been reported previously, poor ventilation, which results in cold draft, is a risk factor for developing mastitis (Schukken et al., 1991).

Further explanations for the present results include a large contribution of contagious pathogens relative to environmental pathogens in the development of the mastitis episodes studied. Due to the large number of subclinical infections with *Staph. aureus* in the herd, the number of mastitis cases with environmental bacteria was relatively low. Since laterality is thought to be mainly a risk factor in developing mastitis episodes caused by environmental pathogens, the great contribution of contagious pathogens to the development of mastitis episodes may have caused a bias. This could explain the low correlation with laterality and that this correlation was not significant. This fact can also partly explain why the distribution of mastitis incidence within the various stages of lactation differs from the findings of other studies (Schukken et al., 1989).

Furthermore, the large number of subclinical mastitis episodes caused by *Staph. aureus* during the laterality recordings may have caused cows to prefer lying on the unaffected side of the udder. In the 8 weeks that followed the laterality recordings, those episodes may have become clinically apparent and thereby treated. This scenario would give the unjustified impression of an increased probability of the upper half of the udder in a lying cow to be affected when a mastitis episode develops.

The Wald-test revealed a significant difference in laterality between cows that developed mastitis and cows that did not. Cows developing mastitis on ei-

ther side of the udder preferred lying on their left side. Since only 48 cows developed a treated mastitis episode in the 8-week period following the laterality recordings, it can be argued that the average laterality fraction of these cows is not representative for all cows developing mastitis.

The analysis of variance showed a significant difference between the SCC values of cows with treated mastitis and those without treated mastitis in three of the five milking tests. This result was confirmed by the  $\chi^2$ -test, which showed a significant difference between the total number of cows with treated mastitis and the SCC values by each SCC category and in three of five monthly milk tests. These results were to be expected, having known the outcomes of earlier studies (Radostits et al., 1994). The analysis of variance did not show significant differences in milk production determined by milking tests from June to October. This result is contrary to the findings made so far (Raubertas and Shook, 1982; Jones et al., 1984; Bartlett et al., 1990; Cassell, 1994; Ózsvári et al., 2001). Furthermore, the  $\chi^2$ -test revealed a significant difference between the occurrence of treated mastitis and cases with  $> 1,000,000$  SCC values on a monthly basis, but no significant difference could be found between the occurrence of treated mastitis and cows falling in SCC categories of  $< 400,000$  and  $400,000-1,000,000$ , respectively. These results gave countenance to the right selection of mastitis treatments and also to the possibility of high incidence of subclinical mastitis cases in the herd.

In summary, there was a significant difference in laterality between cows that developed mastitis and cows that did not. In cows that developed mastitis, an increasing left laterality fraction was correlated with the increased probability of a right-sided mastitis episode. However, the large variation of the data might contribute to some failing significant relationships. Chronic intramammary infections with *Staph. aureus* may have influenced the results in this study. At the same time, significantly more cows with clinical mastitis had SCC higher than that of cows with no clinical mastitis. A significant correlation between the numbers of cows treated with mastitis and  $\text{SCC} > 1,000,000$  was also detected. Furthermore, there was no statistically significant difference in milk production between the two groups.

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