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THE INCIDENCE OF MASTITIS TREATED WITH ANTIBIOTICS IN LARGE-SCALE HUNGARIAN HOLSTEIN-FRIESIAN DAIRY FARMS

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Treated mastitis episodes at large Hungarian dairy farms were studied to determine the distribution of mastitis treated with antibiotics among quarters of the udder. Data were detailed records of all mastitis episodes that occurred during 1976 lactations in Farm A infected with Staphylococcus aureus (from May 1995 through July 1998) and 808 lactations in Farm B free from S. aureus (from January 1999 through March 2001). The distribution of treated quarters was compared with mathematical expectations based upon a random distribution in the case of Farm A. Results on mastitis incidences for different lactation stage groups showed an increasing incidence within subsequent lactation stage groups in Farm A. In contrast, in Farm B the mastitis incidence for lactation stage group between 35 and 100 days was the highest, but beyond 100 days the incidence decreased and reached the lowest value. Results gave strong evidence that the four quarters within the udder are not distributed randomly with respect to naturally occurring episodes of treated mastitis. More episodes than expected occurred in which only one or all four quarters were treated. Fewer episodes than expected with two or three treated quarters were observed. In both farms, the mastitis rate for rear quarters was higher than for front quarters, and the incidence of right quarter mastitis episodes was higher than that of left quarter mastitis episodes.

Key words: Dairy cattle, mastitis, incidence, antibiotics

Mastitis is an economically important aspect of milk production. It is generally accepted that mastitis is one of the most costly diseases to affect the dairy herd (Ward and Schultz, 1974; Horváth, 1982; Booth, 1988; McInerney and Turner, 1989).

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Reports on distribution of clinical mastitis within the bovine udder have revealed that the incidence of clinical mastitis on 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 (Batra et al., 1977; Adkinson et al., 1993; Barkema et al., 1997). Walsh (1985) reported a different incidence of clinical mastitis between right and left quarters in a herd with a one-sided milking parlour. Zadoks et al. (2001) found the rate of *Staphylococcus aureus* infection higher in right quarters. Deviation from expectation of observed mastitis incidence on quarter level could be attributed either to the contagiousness of microorganisms or to differences in risk factors between the quarters of the bovine udder in developing clinical mastitis (Barkema et al., 1997).

The objective of the present study was to investigate the distribution of mastitis treated with antibiotics on quarter level.

Materials and methods

Animals and housing

This study was conducted at two large Hungarian dairy farms. The herd on Farm A and Farm B consisted of 950 and 440 Holstein-Friesian cows, respectively. Farm A was infected with *S. aureus*, while Farm B was free from *S. aureus* infection. The cows were individually numbered for identification in both farms. In Farm A the lactating herd was divided in six production groups, each housed in a free-stall loose housing system. The cubicles 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. Farm B consisted of 7 free-stall loose housing sheds with paddocks.

In both farms the animals were fed three times during the day in a feed bunk along the open side of the shed. The feed consisted of a mixture of silage, alfalfa and soya completed with concentrate according to production level. Hay and water were available *ad libitum*. Milking times started approximately at 05:00 a.m. and 16:30 p.m. The production groups were milked separately in Farm A through two Alfa Laval double-sided herringbone milking parlours with 24 stands each, and in Farm B through 4 Full Wood milking parlours with 12 stands each. Iodine-based post-milking teat dips were used routinely in the farms.

In Farm A, the mean age of cows was 55 months during the study. In 1997, the mean yield per lactation was 8720 kg of milk with 271 kg fat. Mean lactation length was 349 days. In Farm B the mean yield per lactation was 7341 kg of milk with 247 kg fat in 2000.

Mastitis recording

Detailed records on treated mastitis were kept in the farms' log. These records included identification, distribution among quarters, date of treatment and type of antibiotic used. A treated case of mastitis was defined as any intramammary antibiotic treatment. A separate case was defined as one in which at least 14 days had elapsed since any previous treatment in the same quarter. Because previous infections may not have been completely eliminated or may have altered the likelihood of a new mastitis episode occurring, the records on treated mastitis were separated into two data sets for analyses. Data set 1 consisted of all treated mastitis episodes in all lactation periods, while data set 2 included only the first treated mastitis episode in each lactation period. Lactation periods analysed in this study were from May 1995 till July 1998 in Farm A and January 1999 till March 2001 in Farm B. Age groups were analysed in Farm A only. Age groups and lactation stage groups were formed according to Table 1. The final data set contained 2344 observations on 1976 lactations in Farm A and 1024 observations on 808 lactations in Farm B. Data were edited for obvious errors, corrected if verification of correct values was possible, and deleted if verification was not possible.

Table 1

Age groups and lactation stage groups

Age group		Lactation stage group		
3	2 to 3 years 3 to 4 years 4 to 6 years > 6 years	1 2 3	0 to 35 days lactation 35 to 100 days lactation > 100 days lactation	

Mastitis incidence on herd level

Since no exact culling data were available on individual cows, the calculation of mastitis incidence was based on the number of lactations started. Mastitis incidence on herd level was calculated. Mastitis incidences for the different age groups and lactation stage groups were calculated for all mastitis episodes in all lactations (data set 1) as well as for the first mastitis episodes in all lactation (data set 2) in both farms.

Mastitis distribution on quarter level

Mastitis distribution on quarter level was analysed by determining treated mastitis frequencies of the quarter configurations involved. Comparison was made between observed frequencies and frequencies that would be expected if the distribution of treated mastitis among the quarters was random in the case of Farm A.

The overall treated mastitis incidence on quarter level, calculated as the total number of quarters having treated mastitis divided by the total number of quarters available, was used to determine expected frequencies of quarter configurations with treated mastitis. Since no detailed records were kept of which quarters were functional on a cow at a particular time, the assumption was made that each cow had four functional quarters. Thus, overall treated mastitis incidence on quarter level might be biased slightly downward.

The algorithm for determining expected frequencies of quarter configurations with treated mastitis used the binomial probability distribution assuming independence of quarters as follows:

Formula 1. Binomial probability distribution assuming independence of quarters (Adkinson et al., 1993)

$$p(y) = \frac{24(p^{y}q^{4-y})}{(4-y)}$$

p = probability of a quarter being treated based upon the overall treated mastitis incidence on quarter level observed for a particular data set;

y = number of quarters treated for a particular mastitis episode on a particular cow (y = 0, 1, 2, 3 or 4);

q = (1 - p) = probability of a quarter not being treated.

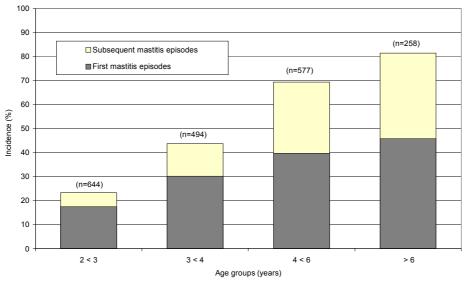
This approach was used for data set 1 as well as for data set 2. Differences between observed and expected frequencies were tested using chi-squared analysis. Observed treated mastitis frequencies for front, rear, left and right quarters were determined and differences were tested using chi-squared analysis.

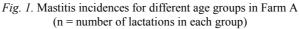
Results

Mastitis incidence on herd level

Mastitis incidence in Farm A on herd level regarding the number of all treated mastitis episodes in all lactations was 977 (49.4%) during the study. The number of first treated mastitis episodes in all lactations was 609 (30.8%). The mastitis incidences for different age groups and different lactation stage groups are shown in Figs 1 and 2, respectively.

Mastitis incidence in Farm B on herd level regarding the number of all treated mastitis episodes in all lactations was 732 (90.6%). The number of first treated mastitis episodes in all lactations was 516 (63.9%). The mastitis incidences for different lactation stage groups are shown in Fig. 3.





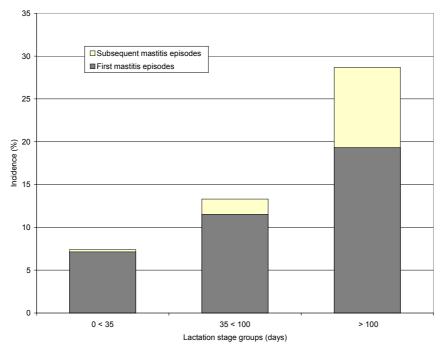


Fig. 2. Mastitis incidences for different lactation stage groups in Farm A (each group consisted of 1976 lactations)

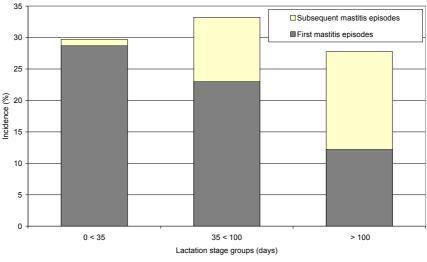


Fig. 3. Mastitis incidences for different lactation stage groups in Farm B (each group consisted of 808 lactations)

Mastitis incidence on quarter level

The observed configurations of treated mastitis episodes in Farm A and B are presented in Tables 2 and 3, respectively.

Expected frequencies for the 16 possible configurations of quarters within a cow with respect to treated mastitis in Farm A were calculated assuming independence (Formula 1). These expected frequencies are presented with corresponding observed frequencies for data sets 1 and 2 in Figs 4 and 5, respectively.

Of all lactations analysed, 1367 lactations were without treated mastitis episodes. The expected number of lactations without treated mastitis episodes was 1443 in data set 1. By the chi-squared test, the difference between observed and expected number of lactations without treated mastitis episodes was significant (P < 0.05).

The observed and expected frequencies of lactations without treated mastitis in data set 2 were 1367 and 1396, respectively. By the chi-squared test, the difference between observed and expected number of lactations without treated mastitis episodes was not significant.

The observed frequencies of treated mastitis for front, rear, left and right quarters in Farm A are shown in Table 4.

Mastitis incidence on udder side level

The observed frequencies of left- and right-sided quarters with treated mastitis and the expected frequencies for the different age groups and lactation stage groups in Farm A are shown in Figs 6 and 7, respectively. Chi-squared test of difference between observed and expected frequencies revealed that the differences within the lactation stage groups and age groups were not significant.

T (] (Data set [*]		
Treated quarters -	1	2	
None	1367	1367	
LF**	194	115	
RF	230	133	
LR	231	154	
RR	252	174	
LF, RF	10	4	
LR, RR	11	6	
LF, LR	7	2	
RF, RR	12	6	
RF, LR	8	2	
LF, RR	9	2	
RF, LR, RR	0	0	
LF, LR, RR	0	0	
LF, RF, LR	0	0	
LF, RF, RR	1	1	
LF, RF, RR, LR	12	9	
Total	2344	1976	

Table 2: Treated mastitis quarter configurations and number of observations in Farm A

Table 3: Treated mastitis quarter configurations and number of observations in Farm B

C	Data set [*]		
Freated quarters -	1	2	
Vone	292	292	
F^{**}	109	88	
RF	107	82	
R	148	102	
RR	180	127	
LF, RF	22	14	
R, RR	36	24	
F, LR	17	7	
F, RR	20	11	
SF, LR	17	12	
.F, RR	18	13	
F, LR, RR	8	4	
.F, LR, RR	3	2	
.F, RF, LR	15	13	
F, RF, RR	13	6	
F, RF, RR, LR	19	11	
otal	1024	808	

*Data set 1 = All treated mastitis episodes in all lactations; data set 2 = first treated mastitis episodes in all lactations. **LF = Left front, RF = right front, LR = left rear and RR = right rear

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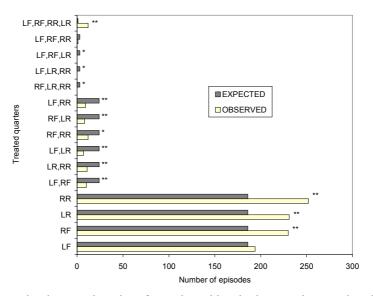


Fig. 4. Observed and expected number of treated mastitis episodes assuming a random distribution among quarters in Farm A. LF = left front, RF = right front, LR = left rear and RR = right rear. Chi-squared test of difference between observed and expected number of episodes within treated quarters group (*p < 0.05; >; **p < 0.01). All episodes in all lactations included (n = 2344)

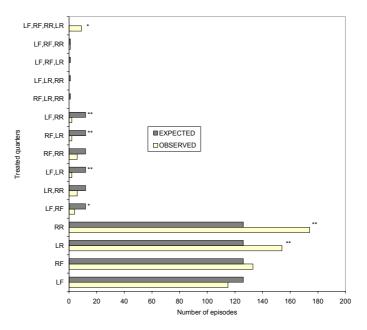


Fig. 5. Observed and expected number of treated mastitis episodes assuming a random distribution among quarters in Farm A. LF = left front, RF = right front, LR = left rear and RR = right rear. Chi-squared test of difference between observed and expected number of episodes within treated quarters group (*p < 0.05; **p < 0.01). First episodes only in all lactations included (n = 1976)

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Frequencies of observed treated mastitis episodes by front, rear, left and right quarters in Farm A

Quartar	Data set [*]		
Quarter	1	2	
Front	0.108 ^{a, c}	0.073 ^a	
Rear	0.121 ^b	0.094 ^b	
Left	0.107 ^a	0.077 ^{a, c}	
Right	0.122^{b}	0.089 ^{b, c}	
Overall	0.114 ^c	0.083 ^c	

^{a, b, c}Frequencies with different superscript letters within a data set are different (p < 0.05); *Data set 1 = All treated mastitis episodes of all lactations (n = 2344), data set 2 = first treated mastitis episodes of all lactations (n = 1976)

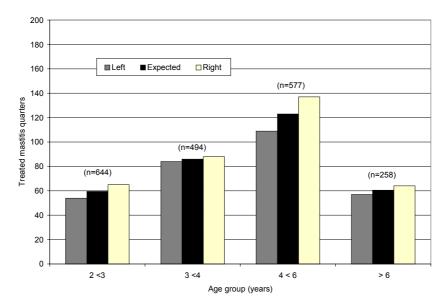


Fig. 6. Observed and expected frequencies of left- and right-sided quarters with treated mastitis for different age groups in Farm A (n = number of lactations in each group)

Discussion

The mastitis incidences on herd level in the present study exceeded the mastitis incidences found by several authors (Dohoo et al., 1983; Erb et al., 1984; Wilesmith et al., 1986; Miltenburg et al., 1996; Barkema et al., 1998). However, the incidence of clinical mastitis on herd level varies significantly

between farms. Schukken et al. (1989) found in a study on the incidence of clinical mastitis on farms with low somatic cell counts in bulk milk, farms with virtually no mastitis, while others had up to 80 cases per 100 cows. The large variation in the incidence of mastitis on herd level suggests that housing and management have a considerable influence on the incidence of the disease (Schukken et al., 1989).

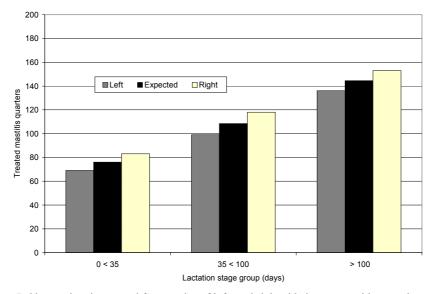


Fig. 7. Observed and expected frequencies of left- and right-sided quarters with treated mastitis for different lactation stage groups in Farm A

Mastitis incidence on herd level can also be influenced by the age distribution within the herd. Results on mastitis incidences for different age groups showed an increasing incidence with the age of the cow. These findings correspond with the results of several studies (Rendel and Sundberg, 1962; Ward and Schultz, 1974; Funk et al., 1982; Oliver and Mitchell, 1983; McInerney and Turner, 1989; Barkema et al., 1998). Also the number of repeated mastitis episodes within one lactation increased with age. Supposedly the rise in the total incidence of udder infections with age is a result of increasing susceptibility to mastitis (Rendel and Sundberg, 1962; Grootenhuis, 1975).

In Farm A mastitis incidences increased within subsequent lactation stage groups. In Farm B the mastitis incidence for lactation stage group between 35–100 days was the highest, but beyond 100 days the incidence decreased and reached the lowest value. These results are contrary to the findings of several authors (Oliver and Mitchell, 1983; Smith et al., 1985; Wilesmith et al., 1986; Schukken et al., 1989; Miltenburg et al., 1996; Barkema et al., 1998), particularly in the case of Farm A. Schukken et al. (1989) and Barkema et al.

case of Farm A. Schukken et al. (1989) and Barkema et al. (1998) found that most of the clinical mastitis cases caused by *E. coli* occurred in early lactation. The frequency of subsequent mastitis episodes increased within subsequent lactation stage groups in both farms, and in Farm B after day 100 of lactation the incidence of subsequent mastitis episodes was higher than that of the first mastitis episodes. The increasing frequency of subsequent mastitis episodes with lactation stage could have been caused by the inadequate treatment of *S. aureus* mastitis episodes (Sol et al., 1997).

Mastitis distribution on quarter level in Farm A was analysed using data sets 1 and 2. More episodes in which only one quarter was involved were observed in data set 1 than would have been expected if quarters were distributed randomly with respect to mastitis status. This result corresponds with the distribution of mastitis amongst quarters found by Adkinson et al. (1993). In data set 2, mastitis episodes with only one quarter treated were also observed more often than would have been expected, except for the left front quarter. Episodes involving two or three quarters were fewer than expected in both data sets 1 and 2. This result is in contrary to findings by Barkema et al. (1997). They found intramammary infections in two or three quarters of the same cow at a higher rate than expected on the basis of a random distribution. Treatment of multiple quarter episodes may have resulted in a cure in some but not all quarters involved. This would result in a shift in distribution from more quarters to fewer quarters being involved in subsequent reoccurrence of previous episodes.

Episodes with all four quarters treated were observed more than would have been expected in data sets 1 and 2. Since a treated mastitis was defined as any intramammary antibiotic treatment, four-quarter treatments of multiplequarter episodes could have resulted in a shift toward episodes with all four quarters treated. It seems likely that in mastitis episodes with three clinical quarters, treatment of all four quarters was often practised. However, the same results were found by several authors (Flock and Zeidler, 1969; Grootenhuis, 1975; Adkinson et al., 1993; Barkema et al., 1997). Fewer episodes with no quarters treated were observed than would have been expected in data sets 1 and 2.

The results of the analyses on mastitis distribution on quarter level gave strong evidence that the four quarters within the udder are not distributed randomly with respect to naturally occurring episodes of treated mastitis. In previous studies the quarter position has been described as a risk factor for the incidence of clinical mastitis and prevalence of subclinical mastitis (Miltenburg et al., 1996; Barkema et al., 1997; Busato et al., 2000). A study by Adkinson et al. (1993) showed that rear quarters had more clinical mastitis than would be expected based on a random distribution. Barkema et al. (1997) stated that the interdependence of quarters is either based on similar risk factors of quarters within a cow or within a herd, or interdependence is based on contagiousness of microorganisms. They corrected prevalence of subclinical mastitis by using intra-class correlation, describing the strength of clustering (Donald, 1993) and found the highest intra-class correlation for *S. aureus* within the herd. Zadoks et al. (2001) found that the exposure to other quarters infected with *S. aureus* and *Streptococcus uberis* within a cow was associated with an increased rate of homologous pathogen. In the present study, the ignoring of the interdependence of quarters within the udder may have caused an underestimation of variance probably due to chronic *S. aureus* infections in Farm A.

Mastitis rates for front, rear, left and right quarters in Farm A were determined for both data sets 1 and 2. Mastitis rate for rear quarters was higher than for front or left quarters when all episodes in all lactations were included (data set 1). Mastitis rate for right quarters was highest in data set 1. When only first episodes in all lactations were included (data set 2), mastitis rate for a rear quarter was higher than for front, left or right quarters. Previous research has indicated that the incidence of clinical mastitis was 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., 1993; Miltenburg et al., 1996; Barkema et al., 1997; Busato et al., 2000). Right-quarter mastitis incidence was higher than left-quarter mastitis incidence in Farm A in both data sets 1 and 2. Several studies did not show a different incidence of clinical mastitis episodes between left and right quarters (Batra et al., 1977; Adkinson et al., 1993). However, Walsh (1985) did report a different incidence of clinical mastitis between left and right quarters in a herd with a one-sided milking parlour. He found that the higher frequency of right quarter mastitis episodes was caused by teat impaction. Barkema et al. (1997) also found that intramammary infections occurred more often in right front quarters than in left front quarters. They suggested that predisposing conditions that are disproportionate between left and right quarters could explain an uneven distribution. A higher prevalence of S. aureus infection in right quarters was also found by Zadoks et al. (2001), but for a limited number of herds the transmission of S. aureus via teat cup liners (O'Shea, 1987) may also explain a higher rate in specific quarters, e.g. right quarters. Right quarters with treated mastitis were most frequent in all lactation stage groups and all age groups in this study. However, the difference between the observed frequencies of left or right treated quarters and the expected frequencies was not significant.

In summary, analyses on the distribution of treated mastitis on quarter level revealed that more mastitis episodes with only one treated quarter were observed than would have been expected if treated quarters were distributed randomly. Fewer episodes with two or three treated quarters were observed than would have been expected. Mastitis episodes with four treated quarters were observed more frequently than expected. Mastitis rate for rear quarters was higher than for front, left or right quarters when first treated mastitis episodes in all lactations were analysed. Incidence of right-quarter mastitis episodes was higher than of left-quarter mastitis episodes. The results of analyses on mastitis distribution on quarter level gave strong evidence that the four quarters within the udder are not distributed randomly with respect to naturally occurring episodes of treated mastitis.

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