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Evaluation of increased milking frequency as an additional treatment for cows with clinical mastitis

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This field study focused on the possible effects of increased milking frequency (milking four times a day in comparison with milking twice a day) on clinical and bacteriological cure rates of clinical, antibiotically treated mastitis cases. Parameters tested were clinical, microbiological and full (cytomicrobiological) cure as well as the development of milk yield after the clinical mastitis episode. Cows from a large dairy herd meeting the study criteria (n=93) were assigned to two treatment groups by a systematic randomization scheme (blocked by body temperature <=or >39·5 °C). Both groups were randomly divided by experimental treatments: a) antibiotic intramammary treatment and milking 2-times a day; b) antibiotic intramammary treatment and milking 4-times a day. Treatments were initiated before the culture results were known. Cows were surveyed and evaluated on days 1–6, 24 and 31. No significant differences between treatment and control groups regarding clinical cure, microbiological cure, full cure and milk production could be established. Applying a 4-times a day milking regime did not lead to any significant effect, either positive or negative. Therefore, the results suggest that milking 4-times a day as a supporting therapy for mild, moderate and severe antimicrobially treated mastitis cases cannot be recommended.

Keywords: Increased milking frequency, mastitis, bacteriological cure rates, antibiotic therapy.

Most therapeutical measures taken in cattle in Germany consist of the treatment of udder health impairments (Krömker, 2006). Among these, milking-out an affected quarter several times a day (increased milking frequency, IMF) is a popular recommendation for the treatment of clinical mastitis (Weigt & Grunert, 1984; Eberhart et al. 1987). IMF is performed to enhance the removal of abnormal secretions, pathogens, toxins and inflammatory mediators contained in the milk of an infected quarter. Although this measure appears to be reasonable, there is little solid evidence to support this routine, and some data even suggest that it can be detrimental (Roberson et al. 2004). Czech research (Opletal et al. 1985) could find no evidence of significant differences in clinical cure rates between IMF alone and intramammary antibiotic administration (IMMA). The increased milking frequency group was milked every 2–3 h daily with an 8-h rest period at night. Yet, this paper was written on the condition that quarters were not subjected to cytobacteriological analysis, and diseased quarters from the IMF group were transferred to the IMMA group after 48 h if no effect could be observed. More recent papers failed to confirm an effect of IMF (Roberson, 1997). When compared with untreated animals with experimentally induced coliform mastitis, IMF (every 4–6 h) in conjunction with oxytocin administration did not shorten the time to clinical or bacteriological cure or the resolution of systemic illness in affected cows (Leininger et al. 2003). Treating mild and severe cases with supporting measures (oxytocin, IMF, anti-inflammatory therapy, fluid therapy) showed inferior effect (lower clinical and bacteriological cure rates, higher recurrence rate and worsening of symptoms) than did antibiotic therapy in conjunction with these supporting measures (Morin et al. 1998). Few papers have been published so far on using machine-mediated IMF as an additional measure to support local or even systemic antibiotical therapy. While an older paper from Germany reported positive

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effects of IMF on the cure rate of antibiotically treated mastitis cases (Stute, 1978), a more recent contribution from the USA (Roberson et al. 2004) showed that IMF applied as the only measure was not effective against mild and moderate mastitis regarding clinical and microbiological cure. The combination of IMF and IMMA tended to keep milk losses lower than IMMA alone would have, but did not improve the recovery after clinical mastitis and resulted in a lower microbiological cure especially for environmental streptococci which led to the conclusion that this measure could not be recommended. The data presented in this American paper rather suggests that IMF triggers the bacterial infection. There is a need for papers dealing with the effects of repeated milkings when performed in larger herds under practical conditions. The present study was designed to determine the effect of repeated milkings [4-times a day (4TD) as opposed to 2-times a day (2TD)] on the clinical and microbiological cure rates of clinical, antibiotically treated mastitis quarters under field conditions.

Materials and Methods

The study took place from January until June 2007 and was performed in a 2000-head dairy enterprise with German Holstein cows in the Free State of Thuringia. Cows were housed in free-stall barns with slatted floors and individual cubicles (rubber mat covered with chaffed straw) and milked on a 2TD basis. Dry cows had access to grazing between May and October of each year. The somatic cell count (SCC) of the herd varied between 150 000 and 200 000 cells/ml. Milking routine included disposal of the pre-milking sample and teat cleaning with dry, disposable paper tissues. After milking, all teats were disinfected using a teat disinfectant (iodine content: 1000 ppm). A solution containing 500 ppm of peracetic acid was used to disinfect the milking clusters between different cows. All cows received a commercial antibiotic preparation at dry-off. Milking personnel identified clinical mastitis cases during their milking routine. When clinical mastitis cases were identified by visual pre-milk check, animals were separated from the rest of the herd immediately after milking and transferred to an extra barn for sick animals. There, careful veterinary examination took place and udder health was categorized according to IDF (1999) recommendations. For the duration of the trial, animals with mild or moderate mastitis cases were treated with cefquinome intramammary, while severe cases (i.e., systemic findings, body temperature >39·5 °C) additionally received s.c. enrofloxacin and 1·1 mg/kg of i.v. flunixin-meglumine as recommended by the manufacturer. Cows without signs of clinical mastitis or with other diseases during the last 14 d before the onset of the trial were excluded from the study. A mastitis episode was considered to be a new one when no flakes were found in the pre-milking sample for at least 5 d prior to the new episode.

Numbered collars and ear tags were used to identify the cows in the herd. After determining which drugs would be used for treatment, each cow included in the study was assigned randomly to one of the two treatment groups (4TD *v.* 2TD). Cows were not stratified any further before this assignment took place. Animals of the trial group were milked at 4TD, cows of the control group at 2TD; both groups were subjected to machine-milking. The first milking after antibiotic application took place after 6 h at the earliest. Assignment to the treatment and control groups was done before the results of the bacteriological analysis were known. The person in charge of the animals examined the animals twice a day for at least 6 consecutive days. This examination comprised the general demeanour, body temperature of the animal, the milk secretion type, impurities encountered in the secretion and the state of the inflammation in the corresponding udder quarters. Before initial treatment began, double samples of quarter foremilk were taken for microbiological analysis. These samples were frozen at −20 °C for up to 4 weeks before processing. At 14 and 21 d after the end of the withdrawal time, another set of quarter foremilk samples was collected. Samples were sent to the laboratory (Heisterbergallee 12, D-30453 Hannover) by mail, cased with cooling pads and kept at 4 °C until microbiological assays and SCC tests were performed. The first jets of pre-milking secretion were discharged, and then two 10-ml samples of milk were collected aseptically (teat tips cleaned previously with 70% ethanol) in two sterile vials from each udder quarter.

Laboratory procedures

Ten microlitres of each milk sample was spread on blood agar plates (5% defibrinated sheep blood, Oxoid, Wesel, Germany). Plates were incubated aerobically at 37 °C and examined after 24 h and 48 h. Provisional colony identification was based on Gram stain, morphology and haemolysis patterns. Numbers of each colony type were also recorded. Representative colonies were then subcultured on blood agar plates and incubated aerobically at 37 °C for a further 24 h to obtain pure cultures. Gram-positive cocci were tested for catalase and coagulase production. A more specific identification of staphylococci was achieved using the coagulase test. Gram-positive, catalase-negative isolates were tested for the CAMP phenomenon, aesculin reaction, growth at 45 °C, and commercial micromethods (API Strep®; BioMérieux, Germany). Gram-negative rods were subcultured on Violet Red Bile agar and were subjected to oxidase and indole reaction tests. Additionally, they were cultured in Triple Sugar Iron Agar and Simmons' Citrate agar. Taking into account the limitations of this method of analysis, the identified bacteria were recorded at the genus or species level whenever possible, whereas unidentified organisms were recorded merely as 'Gram-negative' or 'Gram-positive'. Some Gram-positive rods that could be identified with other simple procedures were identified and recorded (e.g., *Corynebacterium* spp.). The

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infection status of a single udder quarter was defined according to the procedures recommended by the German Veterinary Association (GVA, 2002) and the National Mastitis Council (NMC, 1999). Intramammary infection (IMI) was diagnosed in this study when >500 cfu/ml of identical micro organisms (>5 cfu/10 µl) could be found in both samples. A milk sample was defined as contaminated when >3 distinct colony types were isolated from it.

Determination of the somatic cell count

SCC was measured in all samples using fluorescence microscopy (Fossomatic 360, Foss Electric, Denmark). In order to ensure the normal distribution of the data, SCC values were transformed into decadal logarithms.

Cure definitions

Outcome variables examined were clinical cure, microbiological cure, full cure and daily milk yield. An animal was considered 'clinically cured' (CC) when the demeanour score had been 0 and no anomalies in regard to udder and secretion had occurred for at least 3 d. 'Microbiological cure' (MC) was certified when the mastitis-causing pathogen of an animal had been absent in both control milk samples. When the health status of the affected quarter had been defined as 'normal secretion' (i.e., SCC <100 000 cells/ml foremilk, no pathogens present) in both post treatment samples, the animal was considered 'fully cured' (FC). Daily milk yield was calculated from individual milk yields as recorded during the last DHI data before the onset of the clinical case and the three subsequent recordings after the mastitis episode.

Statistical analysis

Logistic regressions and Fisher's Exact Tests (SPSS 15.0, Chicago IL, USA) were used to analyse the effect of treatment group or culture result on the dichotomous outcome variables CC, MC and FC. The statistical unit was the cow. If a cow had more than one infected quarter, the cow was classified as 'cured' if all affected quarters met the definitions for 'cure'. The effect of treatment on milk production was analysed using repeated measures ANOVA with factors 'number of lactation' as fixed (first, second, and other) and 'days in milk' as random effects. Discrete dependent variables, e.g., time passing until CC or duration of the fever were evaluated by means of mixed linear models with blocking factors for the parameter 'number of lactation' included as fixed and 'days in milk' as random effects. Statistical significance was assumed at $P \leqslant 0{\cdot}05$.

Results

Descriptive analysis

A total of 107 clinical mastitis episodes from 93 cows (97 quarters involved) entered the analysis. Cows presenting with 1, 2 and 3 mastitis episodes numbered 82, 8 and 3 respectively. The shortest interval between two clinical mastitis episodes was 28 d (median interval=41 d). Animals with repeated clinical mastitis cases on one quarter were excluded from the trial. The distribution of cases in relation to lactation number was: 22% in the 1st lactation, 33% in the 2nd, and 45% in the >2nd lactation. Days in milk ranged from 1 to 510 d. No significant differences ($P>0{\cdot}5$) were encountered for these factors between the two groups. Twelve of all mastitis cases (11%) were diagnosed as severe mastitis involving fever.

Table 1. Culture results from clinical mastitis samples (quarter foremilk) by organism

Organism	*n*	%
No growth	38	35·5
Streptococcus uberis	21	19·6
Streptococcus dysgalactiae	14	13·1
Echerichia coli/Coliforms	13	12·1
Enterococcus spp.	5	4·7
Staphylococcus aureus	5	4·7
Arcanobacterium pyogenes	3	2·8
CNS	3	2·8
Staph. aureus+Str. uberis	2	1·9
Coliforms+Enterococci	1	0·9
Corynebacterium bovis	1	0·9
Pseudomonas spp.	1	0·9
Total	107	100

Results of the microbiological analysis

In 38 of 107 cases no positive bacteriological findings were made. Two different pathogens were present in three secretion samples (Table 1). It was not possible to establish any significant differences ($P>0{\cdot}7$) in the different cure levels (CC, MC, FC) between the two trial groups (2TD and 4TD) with regard to microbiology, regardless of the taxonomic level. However, the type of pathogen involved had a stronger influence on the cure rate than the kind of treatment given. CC, MC and FC rates are shown in Tables 2 and 3. Many relations between parameters did not yield significant differences: a) shorter milking intervals on the duration of the fever and the return of the milk secretion to its physiological state, b) severe mastitis cases (evaluated separately) compared with the treatment groups, c) daily milk yield (both before the onset of the clinical mastitis and in post-event periods) (Table 4).

Discussion

Although the supposed usefulness of IMF is widespread in many publications, few papers actually deal with the influence of IMF on the cure rates of clinical mastitis cases. Since mastitis cure definitions and trial designs—IMF as only therapy or as an additional therapeutic

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Table 2. Cow clinical, microbiological and cytobacteriological cure by treatment and organism category

Milking frequency, /d	2	4	2	4	2	4
Culture category	CC†	CC	MC‡	MC	FC§	FC
Coliform	100% (4/4)	44% (4/9)	50% (2/4)	78% (7/9)	25% (1/4)	22% (2/9)
Mixed	100% (1/1)	50% (1/2)	0% (0/1)	50% (1/2)	0% (0/1)	50% (1/2)
No growth	83% (15/18)	75% (15/20)			44% (8/18)	35% (7/20)
Other	88% (7/8)	80% (4/5)	38% (3/8)	40% (2/5)	0% (0/8)	40% (2/5)
Streptococci	59% (13/22)[a]	83% (15/18)[b]	59% (13/22)	61% (11/18)	36% (8/22)	33% (6/18)
Total	75% (40/53)	72% (39/54)	57% (30/53)	63% (34/54)	32% (17/53)	33% (18/54)

† CC = clinical cure
‡ MC = microbiological cure
§ FC = full (cytomicrobiological) cure
Different superscripts within a row and cure category denote different means ($P<0{\cdot}05$)

Table 3. Cow clinical, microbiological and cytobacteriological cure by severity of mastitis

Milking frequency, /d	2	4	2	4	2	4
Mastitis category	CC†	CC	MC‡	MC	FC§	FC
Mild or moderate	78% (38/49)	74% (34/46)	55% (27/49)	61% (28/46)	33% (16/49)	30% (14/46)
Severe	50% (2/4)	63% (5/8)	75% (3/4)	75% (6/8)	25% (1/4)	50% (4/8)

† CC = clinical cure
‡ MC = microbiological cure
§ FC = cytobacteriological cure

Table 4. Post-mastitis event periods compared with pre-treatment milk yields

	Milk LSM† ± SE				
Treatment	Post mastitis	30 d	60 d	90 d	120 d
IMMA+ IMF‡	33·3 ± 1·1	32·0 ± 1·9	30·5 ± 1·1	29·2 ± 1·3	26·4 ± 1·1
IMMA	34·5 ± 1·1	32·7 ± 1·9	31·9 ± 1·1	30·3 ± 1·3	26·5 ± 1·1

† LSM = Least-square means ± SE at day in milk (DIM) adjusted for lactation group and DIM category
‡ IMMA = Intramammary antibiotics; IMF = increased milking frequency

measure—vary, the few existing publications cannot be compared properly. Furthermore, sample sizes typically were too small, and research was carried out in single herds at a time only. This is also the case in the present study. Thus, only hypotheses can be derived from it, whose veracity must be scrutinized in future investigations with more herds and a larger number of animals.

For the distribution of the encountered pathogens, the results of the present survey are comparable to those of other studies (Morin et al. 1998; Giovannini & Zecconi, 2002). Cure rates in relation to the array of pathogens also correspond with similar publications (Roberson et al. 2004).

In the present study, no effect of IMF on mastitis cure rates as a supporting measure could be established, when compared with regular 2TD milking routine. Morin et al. (1998) and Roberson et al. (2004) reached similar conclusions. However, Roberson et al. (2004) additionally observed that the combination of IMMA and IMF had been detrimental for the cure of mastitis cases caused by environmental streptococci. In this study, IMF was applied to the mastitic quarters at least 2 h apart during the day for 3 d in addition to the 2 regular milkings in the parlour for a total of 6 milk-outs per infected quarter per 24-h period. Reduced CC and MC rates were observed in the animals treated with this combination in comparison with the untreated control group. They also found that the manifestation of a CC was prolonged in this treatment group (IMMA+IMF). The explanation provided by the authors was that IMF would stimulate bacterial growth within the udder, a phenomenon that they could confirm in the case of environmental streptococci. The development of bacterial counts right after treatment was not monitored in the present study. However, no detrimental effects of IMF with regard to the cure of mastitis provoked by specific pathogen groups were encountered.

As far as the development of milk yield after an episode of clinical mastitis is concerned, Roberson et al. (2004) found a tendency for milk production loss to be less in the IMF+IMMA group than in the IMMA group. Our data did not show a similar tendency (Table 4). No significant differences regarding the development of milk yield between both therapy groups were noted, but milk yield reduction was less than that reported by Schukken et al. (2009).

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Apart from the role of additional milking as a supporting therapy, the overall effect of repeated milkings on the cell counts is judged differently. While some papers could demonstrate a short-term increase in SCC related to increased milking intervals (Hamann & Gyodi, 1999, 2000), others observed a reduction of SCC (Klei et al. 1997; Hamann et al. 1998). Besides influencing mechanisms of lactation physiology, increased milking frequencies also affect infection-related factors, e.g., removal of micro-organisms and their metabolites, as well changing the mechanical load of the teats (Van der Iest & Hillerton, 1989).

Conclusion

The present paper evaluates the effect of increased milking frequencies as applied by a mechanical milking system in a large dairy herd with a limited number of clinical mastitis cases. No effect could be found when cows with clinical, antibiotically treated mastitis were milked 4-times instead of 2-times a day. Thus, IMF showed no influence on the clinical, the microbiological, the full cure rate, or on the post-mastitis milk yield. The data produced in this study suggest that the combined therapy of IMF (4TD) with IMMA to treat slight, moderate and severe cases of clinical mastitis cannot be recommended. This statement supports the results of a series of recent studies that also failed to find any positive influence of additional IMF on the cure rates of clinical mastitis in dairy cows.

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