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Original Article

Risk factors associated with bacteriological cure, new infection, and incidence of clinical mastitis after dry cow therapy with three different antibiotics

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Factors affecting bacteriological cure rates (BCR) and new intramammary infections (IMI) during the dry period as well as clinical mastitis (CM) during early lactation were investigated in 414 German Holstein dairy cows receiving dry cow therapy. Cows were treated with either benethamine benzylpenicillin (300,000 IU), penethamate hydriodide (100,000 IU), and framycetin sulphate (100 mg, n = 136), or cefquinome (150 mg, n = 135), or benzathine cloxacillin (1,280 mg, n = 143). Overall BCR, IMI, and CM at parturition were 86.4%, 20.7%, and 4.3%, respectively. The three antibiotic treatments differed only in BCR, with cloxacillin yielding better results than the others. Udder quarters from cows with > 4 lactations had a higher risk of IMI and CM at calving. Chronic changes in udder tissues were linked to a lower BCR and were associated with a higher risk of CM during early lactation. The risk of CM at calving was higher in udder quarters with unspecific or subclinical mastitis before drying off. In conclusion, with antibiotic dry cow therapy, age and health status of the udder appear to be major determinants of IMI and CM during the dry period and early lactation, while BCR was associated with the antibiotic type and udder tissue status.

Keywords: dry cow treatment, risk factors, treatment success

Introduction

In dairy cows, the nonlactating or dry period is important because persisting intramammary infections (IMI) acquired during the preceding lactation and new infections contribute to the occurrence of subclinical and clinical mastitis (CM) during subsequent lactation with negative effects on milk yield [14].

At present, the intramammary administration of long acting antibiotics either alone or in combination with internal teat sealers is the most effective measure to prevent new infections and cure existing IMI [13]. Therefore, the intramammary application of long-acting antibiotics is an essential element in the drying off routine of many dairy operations, and has been recommended worldwide for many years [9,20].

Besides the type of treatment at drying off, various other factors affect udder health during and after the dry period, *e.g.* management aspects (housing conditions of the dry cow, farm-specific bacteriological status) and individual animal features such as teat condition and integrity of the teat canal, somatic cell count (SCC), number of lactation, milk yield, and body condition [10,14,15]. It is important to identify those factors which may affect udder health in order to monitor and control risk groups. Furthermore, predominate mastitis pathogens vary between countries which suggests the need to generate local data when assessing treatment regimens [5].

The primary objective of this study was to investigate factors in north Germany affecting new infections and bacteriological cure rates (BCR) and incidence of CM following dry cow therapy with three different antibiotics treatments. In addition to analyzing factors including udder quarter, composite milk SCC, and mastitis category before drying off, special emphasis was placed on clinical parameters (teat end callosity, udder palpation score) which can easily be determined in practice to identify animals at risk.

Materials and Methods

Dairy farms

The investigation was performed as a field study on 13

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dairy farms in Lower Saxony, Germany from January 2005 until June 2006. More than 95% of the cows were of the German Holstein (Black Pied) breed. The average annual milk yield was 8,172 kg (range: 6,000 to 10,400 kg). The average annual bulk milk SCC was 242,000/mL (range: 160,000 to 379,000/mL).

Animals

Late pregnant cows with at least one calving were eligible as experimental animals. Enrolled cows suffering from any general symptoms or from CM during the sampling period before drying off were excluded from the study (n = 55). Of the initial 469 cows eligible for the study, data from 414 cows with 1,633 udder quarters were analyzed.

Milk sampling and examination samples

Foremilk udder quarter samples were taken three times at weekly intervals before drying off. After the third milk sampling, all cows were treated with long-acting antibiotics and then dried off. Beginning at three to ten days after calving, three milk samples were taken at weekly intervals. This procedure was repeated at 60 days postpartum. During the dry period and early lactation up to 60 days postpartum, all CM cases were recorded.

Bacteriological examinations were performed in the milk laboratory of the Institute for Food Quality and Food Safety at the University of Veterinary Medicine Hannover (Germany) according to the guidelines of the National Mastitis Council for isolating and identifying mastitis pathogens [19]. The SCC was determined in the clinical laboratory of the Clinic for Cattle at the University of Veterinary Medicine Hannover using a Fossomatic Series 400 (Foss Elektrik, Germany).

Based on the results of the three weekly milk samples, the udder quarters were distributed into four mastitis categories as follows [2]: normal secretion (SCC $\leq$ 100,000/mL, no infection); latent infection (SCC $\leq$ 100,000/mL plus infection); unspecific mastitis (SCC $>$ 100,000/mL, no infection); and mastitis (SCC $>$ 100,000/mL plus infection). An udder quarter was considered infected when the same mastitis pathogen was isolated in two out of three milk samples. An increase in the SCC was considered to have taken place when the SCC was $>$ 100,000/mL in at least one milk sample. After parturition, the first sampling was excluded from the SCC evaluation due to the colostral character and therefore, high SCC of the milk secretion. The term subclinical mastitis was used when milk secretions from udder quarters allocated to the category "mastitis" indicated no macroscopic changes.

Treatment groups

Cows were randomly distributed among three treatment groups administered the following long-acting antibiotics: 1) benethamine benzylpenicillin (300,000 IU), penethamate hydriodide (100,000 IU) and framycetin sulphate (100 mg) (BPF; Boehringer Ingelheim Vetmedica, Germany) (n = 136); 2) cefquinome (150 mg; Intervet Deutschland, Germany) (n = 135); 3) benzathine cloxacillin (1,280 mg; Pfizer Pharma, Germany) (n = 143). All four quarters of every cow were treated with the same antibiotic therapy.

Further investigations and data collection

Before drying off, udder quarters of all cows were palpated and scored as described previously [23]. In addition, the teat end score was determined [21] and the body condition was recorded using the body condition score (BCS) on a five-point scale with 0.25 increments [11].

The number of lactations (related to the expected calving), the length of the dry period, the milk yield of the last milk quality control test day, and the SCC in composite milk samples of the last three milk quality control test day results before drying off were recorded.

Statistical analysis

All statistical analyses were performed on an udder quarter basis using the statistic program SAS (version 8.2; Statistical Analysis System, USA). Initially, univariate analysis was performed to test the effect of each independent variable on various dependent variables applying Chi-square or Fisher's exact tests [24].

The dependent variables were defined as follows: apparent BCR = proportion of infected udder quarters at drying off, where the same udder pathogens could not be isolated at calving; apparent new infection rate (%) during the dry period = proportion of udder quarters with mastitis pathogens at calving which had not been present before drying off; incidence of CM postpartum (%) = proportion of udder quarters with CM immediately postpartum; incidence of CM up to 60 days postpartum = proportion of udder quarters with CM in the period from calving up to 60 days postpartum (cases immediately postpartum not included).

The independent variables tested were: antibiotic treatment, number of lactation (following the dry period), BCS, duration of the dry period ($\leq$ 63 days, $>$ 63 days), milk yield of the last test day ($\leq$ 20 kg, $>$ 20 kg), teat end score (no or small smooth ring, rough ring), udder palpation score, udder quarter localization, geometric mean of udder quarter SCC before drying off, geometric mean of SCC in composite milk samples of the last three test days before drying off, and udder quarter mastitis category.

Independent variables having an effect with a p value $<$ 0.3 were included in logistic regression models using the SAS procedure GLIMMIX and a logit link function [27]. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each variable in the final models. To account for nesting effects, herd and cow were included as random effects. In the final models, the variable mastitis

category had only three levels instead of four, because due to the low number of latent infections, subclinical mastitis and latent infections were combined. In the final models, statistical significance was declared at a p value < 0.05.

Results

Mastitis pathogens

Before drying off, 61.1% (n = 997) of 1,633 milk samples were bacteriologically negative and 38.9% (n = 636) were bacteriologically positive. After calving 25.7% (n = 420) of 1,633 milk samples were bacteriologically positive as were 32.6% (n = 453) of 1,388 milk samples 60 days postpartum. Table 1 shows the proportion of isolated mastitis pathogens during the three sampling periods. After calving, 229 infections with coagulase negative staphylococci (CNS) and 95 infections with coryneforms were noted. Of those, 34 infections with CNS (14.9%) and 34 (35.8%) infections with coryneforms were from udder quarters which were positive for the same bacteria group before drying off.

Apparent BCR during the dry period

Of 636 infected udder quarters before drying off, 87 remained positive with the same mastitis pathogen after calving, yielding an apparent BCR of 86.3%.

Udder quarters that had been treated with cloxacillin or from cows either with more than four lactations or with an udder palpation score ≤ 2, had a higher chance of being cured than the other respective udder quarters (Table 2).

Table 1. Distribution of isolated mastitis pathogens in milk secretions classified as bacteriological positive*

Pathogen	Before drying off		After calving		60 days postpartum	
	n	%	n	%	n	%
Coagulase negative staphylococci	196	30.8	229	54.5	123	27.2
Staphylococcus aureus	34	5.4	8	1.9	8	1.8
Streptococcus dysgalactiae	9	1.4	1	0.2	2	0.4
Streptococcus uberis	23	3.6	18	4.3	14	3.1
Coliforms	3	0.5	26	6.2	1	0.2
Arcanobacterium pyogenes	0	0.0	2	0.5	1	0.2
Coryneforms	278	43.7	95	22.6	212	46.8
Enterococci	29	4.6	7	1.7	12	2.7
Mixed infections	56	8.8	22	5.2	74	16.3
Other	8	1.3	12	2.9	6	1.3
Total	636	100.1[†]	420	100.0	453	100.0

*At least two positive findings in three milk samples taken at weekly intervals. [†]Due to rounding up, figures do not add up to 100.

Table 2. Odds ratios (ORs) and confidence intervals (CIs) of factors with significant effects on the bacteriological cure rate during the dry period in the final logistic regression model

Independent variable	Class	OR	95% CI	p value
Antibiotic	BPF*	3.3205[a]	1.2509 ~ 8.8143	0.0079
	Cefquinome	4.5195[a]	1.6916 ~ 12.0751	
	Cloxacillin	1.0000[b]		
Number of lactation	= 2	7.3368[a]	2.1023 ~ 25.6051	0.0078
	3 + 4	4.8979[a]	1.4650 ~ 16.3752	
	> 4	1.0000[b]		
Udder palpation score	Finely granular (1)	0.2439[a]	0.0702 ~ 0.8465	0.0023
	Coarsely granular with solitary lumps (2)	0.1424[a]	0.0431 ~ 0.4708	
	Nodular, indurations (3)	1.0000[b]		

*BPF: benethamine benzylpenicillin, penethamate hydriodide and framycetin sulphate. [a,b]ORs with different letter superscripts are statistically significantly different. The OR describes the risk for udder quarters of not being cured.

Apparent new infection rate during the dry period

The overall apparent new infection rate was 20.7% (n = 338). Udder quarters from cows with fewer than four lactations, with a SCC ≤ 400,000/mL, and with subclinical mastitis had a lower risk of new infection than the other respective udder quarters (Table 3). Furthermore, right hind quarters had a higher risk of new infection.

Incidence of CM

Udder quarter incidence of CM at parturition was 4.3% (n

Table 3. Odds ratios (OR) and confidence intervals (CI) of factors with significant effects on new infections during the dry period in the final logistic regression model

Independent variable	Apparent new infection rate during the dry period			
	Class	OR	95% CI	*p* value
Number of lactation	= 2	0.5892[a]	0.3544 ~ 0.9796	0.0019
	3 + 4	0.4438[a]	0.2832 ~ 0.6954	
	> 4	1.0000[b]		
Udder quarter localization	Rear left	0.8740[a]	0.6305 ~ 1.2115	0.0264
	Rear right	1.3885[b]	1.0123 ~ 1.9044	
	Front left	1.0285[a,b]	0.7568 ~ 1.3978	
	Front right	1.0000[a]		
Mean udder quarter SCC* before drying off	≤ 100,000/mL	0.3404[a]	0.1904 ~ 0.6084	0.0007
	101,000 ~ 400,000/mL	0.6770[b]	0.4990 ~ 0.9184	
	> 400,000/mL	1.0000[c]		
Mastitis category	Subclinical and latent infection	0.3214[a]	0.1619 ~ 0.6379	0.0001
	Unspecific mastitis	0.6518[b]	0.3365 ~ 1.2628	
	Normal secretion	1.0000[b]		

*SCC: somatic cell count. [a,b,c]ORs with different letter superscripts are statistically significantly different. The OR describes the risk for udder quarters of acquiring a new infection.

Table 4. Odds ratios (OR) and confidence intervals (CI) of factors with significant effects on the incidence of clinical mastitis (CM) during the dry period and from calving up to 60 days postpartum in the final logistic regression model

Independent variable	Class	Incidence of CM during the dry period			Incidence of CM from calving until 60 days postpartum		
		OR	95% CI	*p* value	OR	95% CI	*p* value
Number of lactation	= 2	0.5892[a]	0.3544 ~ 0.9796	0.0019	No significant effect		
	3 + 4	0.4438[a]	0.2832 ~ 0.6954				
	> 4	1.0000[b]					
Body condition score	< 3.0	Not included into the model*			0.8471[a,b]	0.0600 ~ 11.9623	0.0083
	3.0 ~ 3.5				7.0823[a]	1.7533 ~ 28.6086	
	> 3.5				1.0000[b]		
Palpation score	Finely granular (1)	No significant effect			0.2561[a]	0.0732 ~ 0.8954	0.0013
	Coarsely granular with solitary lumps (2)				0.1912[a]	0.0575 ~ 0.6358	
	Nodular, indurations (3)				1.0000[b]		
Mastitis category	Subclinical and latent infection	2.5080[a]	0.9639 ~ 6.5259	0.0001	Not included into the model*		
	Unspecific mastitis	3.9001[b]	1.5457 ~ 9.8406				
	Normal secretion	1.0000[a]					

The OR describes the risk for udder quarters of developing CM. [a,b]ORs with different letter superscripts are statistically significantly different.
*Only variables with $p < 0.3$ in the univariate analysis were included in the final logistic regression models.

= 70). Udder quarters from older cows (> 4 lactations) and with unspecific mastitis before drying off were at a higher risk of developing CM at calving (Table 4).

Udder quarter incidence of CM up to 60 days postpartum was 3.0% (n = 41). Udder quarters from cows with a BCS of 3.0 to 3.5 before drying off had a seven-fold higher risk of developing CM than those from cows with a BCS < 3.0 and > 3.5. Furthermore, the risk of CM was lower in udder quarters with a palpation score ≤ 2 at drying off (Table 4).

Discussion

The most frequently isolated pathogens before drying off, at calving, and 60 days postpartum, were CNS and *Corynebacterium* spp., which is in accordance with other study results [13,22]. The majority of CNS (~ 85%) and coryneform infections (~ 64%) found at calving were new infections during the dry period. It is of interest to note that even with antibiotic dry cow treatment, the highest new infection rates were calculated for CNS (13.1%) and coryneforms (4.3%), whereas these were below 2% for major pathogens (data not shown). Given the limited temporal effectiveness of dry cow formulations [18], this would suggest that many new infections with minor pathogens apparently occurred at the end of the dry period, and that antibiotic dry treatment is of limited value to prevent those new infections.

In this study, all cows were treated at drying off. The cloxacillin formulation was associated with a higher likelihood of cure compared with cefquinome or benethamine benzylpenicillin-penethamate hydriodide-framycetin sulphate (BPF). One reason for the difference in treatment efficacy could be the galenical preparation. The cloxacillin formulation used in this study was dynomilled, *i.e.* super-finely and equally ground, which increases bioavailability and has positive effects on the inhibitory concentration in udder tissue [25]. However, the cefquinome product was also micronized and high levels with uniform diffusion within all areas of the udder were achieved following intramammary application [12]. In another study, no significant differences between cefquinome and dynomilled cloxacillin in terms of cure of IMI caused by major pathogens were identified [6]. In the present study, approximately 90% of the bacteria isolated before drying off were Gram-positive and only 0.5% were coliforms. Therefore, the Gram-negative component of cefquinome and also that of BPF were apparently secondary as far as cure rate was concerned.

Older cows had a higher risk of new infection during the dry period and of CM at calving supporting the findings by other researchers [10,14,16]. Age-related anatomical changes of the teat with negative effects on local resistance (*e.g.* increase in diameter of the teat canal, diminished function of the teat canal sphincter) and reduced general resistance with increasing age are possible reasons for this increased risk of infection [10,14,16]. Considering the cure rate during the dry period, it was surprising that older cows had a lower risk of having no bacterial cure (BC) than younger cows. The reason for this finding is not clear and disagrees with the results obtained in a previous study [7] where a decreasing cure rate during the dry period with increasing age was found.

Cows with the desired body condition (3.0 to 3.5) had a higher risk of CM during early lactation than over-conditioned cows (> 3.5). Obviously, deviations of BCS at drying off do not seem to play a major role in udder health during early lactation. Valde *et al.* [26] described animals in low infected herds as having a lower BCS before drying off and during the first month of lactation than animals in high infected herds.

An unfavourable udder palpation score as a marker of chronic udder tissue changes was linked with an increased risk of no BC as well as an increased risk of CM during early lactation. Chronic tissue changes are most often sequelae of repeated mastitis episodes with a decreased likelihood of bacteriological and cytological cure following treatment. Even though the self-cure rate during the dry period is higher than during lactation [17], it appears that this increased self-cure capability is limited by the condition of the udder tissue. A lower BCR and lack of elimination of inflammation in chronic cases might then cause a higher risk of CM during early lactation either from persistent or new infections. In accordance with our results, Barkema *et al.* [4] reported that the cure rate of *Staphylococcus aureus* infections was dependent upon the degree of chronic tissue changes. Cows with nodular udder tissue had a lower BCR than cows with lesser changes. Therefore, the udder palpation score seems to be a suitable parameter for detecting cows with a low tendency of self cure during the dry period and therefore a higher risk of CM.

A higher risk of new infections during the dry period was found in right hind quarters compared to right front and left hind udder quarters which could be due to the unfavourable hygienic conditions for this udder area. In addition, due to the shorter length of the hind teats with a corresponding shorter teat canal, the defence potential in the hind quarter could be decreased [16]. In other studies, higher incidences of mastitis and higher SCC in hind quarters compared to front quarters were also described [1,3]. When the right and left udder quarters were compared, the right hind quarters were affected by higher SCC.

The parameter quarter SCC and quarter mastitis category are used to describe the health status of the udder. Considering the SCC and mastitis category together, our results suggest that the risk of new infection during the dry period is especially high in udder quarters with inflammation (high quarter SCC) but without infection (unspecific mastitis). It is of interest to note that udder quarters with subclinical

mastitis and unspecific mastitis have a higher risk of CM at calving and during early lactation. In a study by Green *et al.* [15], cows with a mean SCC $\geq$ 200,000/mL before drying off had higher SCCs in the subsequent lactation. Dingwell *et al.* [8] noted that the risk of new infection during the dry period was 1.9 fold higher for cows with a mean SCC of $>$ 200,000/mL compared to cows with a lower SCC.

In conclusion, with antibiotic dry cow treatment, the number of lactations, the health status of the udder quarter based on quarter SCC (but not composite milk SCC), microbiology, and udder tissue status seem to be the major determinants of new infection and CM during the dry period and early lactation, while BCR was associated with the type of antibiotic treatment and udder tissue status.

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