Comparison of a non-antibiotic treatment with an antibiotic treatment of chronic mastitis

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Abstract

A non-blinded, positively controlled trial was conducted to evaluate the efficacy of an alternative, non-antibiotic therapy with Pyrogenium® to reduce ineffective antibiotic usage in the treatment of non-severe clinical mastitis (CM) in cows with longer lasting udder diseases. The solely treatment with Pyrogenium® (6 times, at a 12 hourly interval and then 4 times in a 24-hour interval) were compared with the reference treatment of solely local antibiotic therapy. The matched field study was conducted on five free-stall dairy farms located in Northern Germany. Cases of mild-to-moderate CM in cows with longer lasting high somatic cell counts in preceding dairy herd improvement test days and with previous CM cases in current lactation (chronic mastitis) were randomly allocated to one of the two treatment groups. A foremilk sample of the affected quarter was taken before treatment and again approximately 7 days, 14 days and 21 days after the end of therapy for cyto-bacteriological examination. Primary outcome was clinical cure (CC). Bacteriological cure (BC), quarter somatic cell count cure (CYC) and CM recurrence within 60 days after the end of treatment were chosen as secondary outcomes although low probabilities of bacteriological cure and quarter somatic cell count cure for selected cows were expected. The study resulted in the following findings: the pathogens mostly cultured from pretreatment samples were *Staphylococcus aureus*, followed by *Streptococcus uberis*. There were no significant differences between the test treatment (EG) in comparison with the reference treatment (CG) regarding all outcome variables (e.g. CC: EG 60.0%, CG 54.7%). Having regard to the selection criteria of cows in this study, the findings indicated that sole treatment with Pyrogenium® in non-severe CM cases may constitute an alternative therapy to reduce antibiotics.

Keywords: recurrent mastitis, clinical cure, bacteriological cure, non-severe clinical mastitis, alternative mastitis therapy

Introduction

Mastitis is still the most costly disease on dairy farms [1] and therefore, the efficacy of mastitis control is important for dairy farmers. According to recent studies [2, 3], up to half of all clinical mastitis cases (51%) have precedent cases, which means that a substantial percentage of all mastitis cases are recurrences. On cow level, in 6% to 40% cases more than one mastitis occurs in one lactation [4, 5]. Chronic mastitis, which have a low probability of bacteriological cure, are responsible for a considerable use of antibiotics, high milk losses and form a large part of the clinical mastitis cases [6]. In order to ensure responsible handling of antibiotics, antimicrobial treatment should be avoided if the likelihood of bacteriological cure appears very low [7]. Due to the importance of recurrent mastitis as an important trigger for unsuccessful antimicrobial treatment, unnecessary use of antibiotics can be prevented by removing animals with incurable mastitis from the herd [8] or by providing non-antibiotic treatment.

Animal welfare is an increasingly concern of society. Thus, the slaughter of pregnant animals was discussed in the European Union and was also restricted in Germany in 2017 (Tiererzeugnisse-Handels-Verbotsgesetz (TierErzHaVerbG), § 4). In the last third of pregnancy, mammals may only be delivered for slaughter if slaughter has been prescribed or ordered in accordance with regulations on epizootic diseases or if it is necessary in individual cases in accordance with veterinary indications and there are no predominant reasons for animal welfare to prevent a delivery for slaughter. This complicates the removal of chronically ill animals from the herd for farmers. The chances of bacteriological cure of an individual animal with chronic mastitis are low, however by reaching a clinical cure, the affected cow at least could be used for milk production until calving.

Therefore the objective of the treatment of chronic mastitis is to reach clinical cure. For this these cows will be treated with antibiotics. But the objective of antibiotic treatment is the bacteriological cure, which cannot be achieved by cows with chronic mastitis. To prevent a useless application of antibiotics and thereby minimize treatment costs, development of antibiotic resistances, withdrawal periods and therefore milk losses, an effective alternative treatment is needed. An opportunity could be an authorised combination of homeopathic remedies treatment without antibiotics. Currently the Pyrogenium Compositum Inject product (in the following text: Pyrogenium®; SaluVet GmbH, Bad Waldsee) often is used in mastitis cases. With regard to the current debate on antibiotic resistances, homoeopathic remedies should be examined as an effective alternative.

The objective of this trial is therefore to investigate the effect of non-antibiotic treatment in cows with chronic mastitis, based on historic milk recording monthly cow somatic cell count data and mastitis history, with regard to the clinical cure and recurrent cases.

Material and Methods

Study design:

Cows with chronic infections, based on historic milk recording monthly cow somatic cell count data and mastitis history, were enrolled to the study (day 0). The affected quarter of cows with clinical mastitis that met the inclusion criteria was aseptically sampled by a herdsperson who had been trained in sample collection. Samples for bacteriology were collected from each affected udder quarter. Subsequently the quarter was treated with one of two treatment regimens. Based on a randomisation list, randomisation and allocation of the animals to a treatment group was carried out and stratified by lactation number group (1st lactation, >1st lactation). Cows of control treatment group (CG) received treatment with antibiotic intramammary tubes as usual on the farm. The resistance situation of the mastitis isolates obtained to the antibiotic active substances used was tested with the Kirby-Bauer method. In 96.6% of all cases, the mastitis pathogens were sensitive to the selected active substances. Animals of examination treatment group (EG) where treated with Pyrogenium® 10 ml subcutaneously 6 times, at a 12 hourly interval and in the following 4 times at a 24 hourly interval. The withdrawal period is determined by the used antibiotic drug in each farm. The withdrawal period of Pyrogenium® is zero days for meat and for milk. Every milking time during the treatment period plus day 7 (+/-2 days) after enrolment, cow and udder were clinically evaluated by a trained herdsperson. Post treatment quarter samples for bacteriology and somatic cell counting (SCC) were taken at 7 (+/-2 days), 14 (+/- 2 days) and 21 (+/-2 days) days post enrolment. Also in the case of a recurrent mastitis in the same quarter, a milk sample was taken for the bacteriology. Effectiveness of treatment was defined as a clinical cure. A clinical cure was defined as no clots in the milk and no systemic sickness symptoms by clinical evaluation at day 7 after treatment ending. The bacteriological cure, the cytological cure and recurrent cases of the same quarter within a period of 60 days post enrolment were also examined. A bacteriological cure was defined as the absence of a pathogen that was identified just before treatment in both post treatment samples (day 14 (+/- 2 days) and day 21 (+/- 2 days)). A maximum of 2 pathogens per sample was allowed for the determination of bacteriological cure. A sample in which more than 2 pathogens were identified was considered as a contaminated sample. A cytological cure was given when a quarter sample had a low SCC (<200.000 cells/ml) at 14 and 21 days after treatment.

Although it was not a "formal" requirement, the study was conducted according to Good Clinical Practices (GCP). This trail was non-blinded, randomised and positively controlled with two treatment groups. It was not possible to blind either the study personnel or the farmers/herdspersons to product administration by virtue of the differences in treatment regime. The personnel at the laboratory culturing for mastitis pathogens was unaware of the treatment given to the quarter being sampled.

Herds:

The field trial was performed from July 2017 until October 2018 on five commercial dairy free-stall farms (155-932 German Holstein cows; average milk yield 10,432-11,642 kg (fat and protein corrected milk, ECM; bulk milk somatic cell count (BMSCC) ≤ 200,000 cells/mL) in Northern Germany, which have the ability to demonstrate likely compliance with the study protocol. In all farms, the cows were kept in free-stall barns and fed with total mixed rations depending on their milk yield and were milked twice a day in herringbone or side-by-side parlours. The farms must be undertaking monthly milk recording and individual cow somatic cell count testing and must have been recording the percentage of new infections, based on cow SCC for at least 12 months. For each participating farm the monthly milk production, BMSCC, milking routine, details of the milking machine (i.e. configuration, size, make, recent service history etc.), mastitis treatment practices, clinical mastitis cases (date, quarter, etc.) and historical data on percentage of new infections based on cow somatic cell count (CSCC) were documented.

Inclusion of cows:

Clinical mastitis was defined as a quarter with any change of milk aspect and was identified by the trained herdsperson. Training and an assessment of sampling ability of herdspersons had been undertaken prior to commencing the study. Clinical evaluation consisted of a classification of severity of disease as Grade 0: normal, Grade 1: mild, only clots in the milk, Grade 2: moderate, symptoms of grade 1 and heat, pain and/or swelling of the udder, rectal temperature <39.5°C and Grade 3: severe, symptoms of grade 2 and symptoms of depression, anorexia, very swollen udder, rectal temperature >39.5°C or <38.6°C. Following identification of clinical mastitis, each animal was assessed for suitability for enrollment in the study. All animals of all parities were eligible for recruitment to the study that had a clinical mastitis case in not more than one quarter, had have three consecutive high CSCC (>400.000 cells/ml) in the three months or at least two mastitis cases per quarter in the current lactation directly before the occurrence of the clinical mastitis, had have four functional quarters free from significant udder, teat and teat orifice lesions before the mastitis case and have been enrolled on a monthly recording of milk yield and SCC. Animals having systemic sickness symptoms (fever, dehydration, anorexia, depressed), clinical evaluated in Grade 3 mastitis, were excluded from the study as well as animals that already have been enrolled before. Animal parity, quarter affected, most recent milk recording milk yield, historic milk recording monthly somatic cell count data, and relevant clinical data were collected. Data either were recorded at cow-side contemporaneously onto data capture forms or retrieved onto data forms from on farm software at the time of treatment.

Laboratory Methods:

All milk samples were collected aseptically and were stored below 8°C until analysis. Ly20, containing boric acid as reserving agent, was used in test tubes [9]. The samples were sent to the microbiological laboratory at the University of Applied Sciences and Arts Hannover (Germany). Microbiological examinations were performed in accordance with the guidelines of the German Veterinary Association [9], which are based on National Mastitis Council recommendations [10]; 10 µl of each milk sample was plated on a quadrant of an aesculin blood agar plate (Oxoid, Germany) and incubated at least for 48 hrs at 37°C under aerobic conditions. By the assessment of Gram staining, morphology of the colonies and cells, hemolysis patterns, aesculin hydrolysis and activity of catalase (3% H2O2; Merck, Germany), an initial evaluation of the grown colonies was performed. Subsequently, several biochemical tests were performed to determine the growing microorganisms. The clumping factor test (DiaMondiaL Staph Plus Kit, Sekisui Virotech, Germany) instead of the coagulase test was used to differentiate presumptive *Staphylococcus* (*S.*) *aureus* from coagulase-negative staphylococci (CNS). Different aesculin-negative streptococci were distinguished by the serological tests for Lancefield Group B (*Streptococcus* (*Sc.*) *agalactiae*), C (*Sc. dysgalactiae*) and G (DiaMondiaL Streptococcal Extraction Kit Sekisui Virotech, Germany). To differentiate between *Sc. uberis* and *Enterococcus* spp., the modified Rambach agar according to Watts, Salmon, and Yancey (1993) was used [11]. Gram-positive, beta-haemolytic and catalase-negative irregular rods with a V-or Y-shaped configuration were identified as *Trueperella* (*T.*) *pyogenes*. Coryneform bacte-

Table 2:. Cure rates for the examination and control group

	Examination group		Control group		p-value
	amount	percentage	amount	percentage	
Clinical cure	45/75	60.0%	41/75	54.7%	0.509
Bacteriological cure	25/62	40.3%	22/45	48.9%	0.378
Cytological cure	7/75	9.3%	10/75	13.3%	0.440
Recurrent cases of clinical mastitis	19/75	25.3%	24/75	32.0%	0.367

ria form small colonies on aesculin blood agar. They are gram-positive and catalase-positive. Both *T. pyogenes* and coryneform bacteria are asporogen. *Bacillus* spp. form large colonies on aesculin blood agar. *Bacillus* spp. are gram-positive, catalase-positive rods and can form endospores. Coliform bacteria are gram-negative and cytochrome oxidase-negative (Bactident oxidase, Merck, Germany) rod-shaped bacteria, which can metabolize glucose fermentatively (OF basal medium with addition of D (+)-glucose monohydrate, Merck, Germany). On Chromocult Coliform Agar (Merck, Germany), *Escherichia* (*E.*) *coli* forms blue colonies under aerobic incubation at 37°C for 24 hrs, and other coliforms form pink-red colonies. *Klebsiella* spp. are immobile during the performance of the OF test. Pseudomonads were identified as gram-negative, catalase-positive and cytochrome oxidase-positive rod-shaped bacteria that break down glucose oxidatively. Yeasts, moulds and *Prototheca* spp. were differentiated microscopically after subculturing on YGC agar (Merck, Germany). Environment-associated, mastitis-causing microorganisms (*Sc. uberis*, *E. coli*, CNS, *Klebsiella* spp., coliform bacteria, yeasts, *Pseudomonas* spp. and *Prototheca* spp.) were recorded as a microbiologically positive result if ≥ 5 cfu/0.01 ml were cultured to reduce bias due to contamination. If two pathogens were cultured, the case was included in the study and both microorganisms were documented. A milk sample was considered as contaminated when more than two pathogens were identified, except in cases where also *S. aureus*, *Sc. agalactiae*, *Sc. dysgalactiae* and *T. pyogenes* were cultured. Then, only the growth of these pathogens was recorded, and the cases were classified as contaminated if the samples contained more than two of these pathogens. The Somascope Smart (Delta Instruments, The Netherlands) was used to determine the quarter SCC by flow cytometry.

Statistics:

The objective of this trial is to investigate the effect of non-antibiotic treatment in cows with chronic infections, based on cow somatic cell count and mastitis history, with regard to the clinical cure. The tested hypothesis was that the CG (standard therapy) resulted in a higher clinical cure rate (95%) than the EG (Pyrogenium®) (80%). If differences in clinical cure rate between treatment groups are assumed to be 15 % (80 vs. 95 %), and the power to be 0.80, a total of 60 animals will be needed per treatment group. Assuming that approximately 10-15% of cows drop out of the trial post, approximately 75 cows were required per treatment group, in total 150 cows with clinical mastitis.

The data were collected in Microsoft Access and Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA). SPSS (SPSS 25.0, IBM Corp., Armonk, USA) was used for the statistical calculations. Normally distributed metric data were statistically analysed using the Student's t-test in order to test the homogeneity of data of the two treatment groups. The nominal, i.e., clinical grade, data were compared in terms of proportions with a χ^2-test (Chi-Square Test). A value of $p<0.05$ was considered as significant.

Although the affected quarter was the unit of observation for the target variables, only one quarter per cow was included and therefore cow and quarter analysis were identical. Clinical cure (CC), bacteriological cure (BC), and cytological cure (CYC) were evaluated with the help of a mixed model logistic regression analysis wherein parity, days in milk (DIM; ≤100, 101–200, ≥201), grade of mastitis (mild/moderate), treatment and pathogen type (streptococci, staphylococci, coliforms, mixed, no growth, and other) were included as fixed effects. The treatment was the main variable of interest in the study. Categorisation of cytological cure was based on the cut-off value of 200,000 cells/mL as mentioned earlier. The full model can be given by:

Logit (BC, CC, CYC, recurrent cases of clinical mastitis) = Lactation + DIM + mastitis severity + pathogen-group + treatment + pathogen-group x treatment + herd (random) + e

Akaike information criterion was used to determine the model quality. The random farm effect was kept as a design variable even though it was not significant in the models.

Table 1: Amount and distribution of mastitis-causing pathogens in 150 mastitis milk samples from cases of clinical mastitis (microbiological culture)

Bacteriological outcome	Number of samples	Percentage (%)
S. aureus	35	23%
no growth	31	21%
Sc. uberis	22	15%
other	20	13%
contaminated	12	8%
mix	12	8%
***E. coli*/coliforms**	9	6%
Sc. dysgalactiae	7	5%
NAS	2	1%

Results

In total, 150 clinical mastitis cases were included in the evaluation, 75 cases of which belonged to the EG and 75 cases to the CG. Six cases were not considered in the evaluation and were disregarded due to false treatment (too short treatment) or missing control samples, which would distort the evaluation. The two test groups were comparable in terms of days in milk (p=0.121), number of lactations (p= 0.426) and distribution of mastitis severity (p=0.200). The microbiological results of the mastitis samples are shown in Table 1. S. *aureus* was the most frequent pathogen (23%), followed by *Sc. uberis* (15%) and Coliforms (6%). In 96 % of all cases, the pathogens treated with antibiotics in CG were sensitive to the active substances used. On average 4.5 days were

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treated and the waiting time for milk was on average 5 days. Cefalexin and Kanamycin, Amoxicillin/Clavulanic acid and Cefquinome were used in particular. In 21% of all cases, no pathogen was found. More than one pathogen (mixed infections) could be isolated in 8% of all cases. The bacterial spectrum varied significantly between the two groups due to the uneven distribution of contaminants (more in CG) and staphylococci (more in EG) (p= 0.004, data not shown). Therefore, we added an interaction term which allowed us to test the pathogen-group specific effect of EG versus CG. Table 2 shows the cure rates allocated to the examination and control group. The bacteriological cure did not differ much between the groups (EG: 40.3%, CG: 48.9%). The difference was not significant neither was the cure rate of the cytological cure (EG: 9.3%, CG: 13.3%), the percentage of recurrent cases per group (EG: 25.3%; CG: 32.0%) nor the clinical cure at day 7 (EG: 60.0%, CG: 54.7%). Using the generalised linear mixed models, no significant differences for cure rates could be established. The null hypothesis mentioned at the beginning of the paper had to be accepted.

Discussion

The main aim of this study was to evaluate the efficacy of a non-antibiotic intramammary treatment with Pyrogenium® (EG) in comparison with a reference therapy with antibiotics (CG) of non-severe CM in cows with longer lasting udder diseases, with special regard to the clinical cure and recurrent cases.

The study was done on five high-yielding dairy farms in Northern Germany and applied to cows with clinical mastitis. Cows were enrolled and then randomly assigned to the control or to the examination group. In a positively controlled trial, the effect of self-cure cannot be determined. The test design was chosen because the farmers involved did not accept a non-treatment of mastitis cows. Nevertheless, the effect of self-cure should be determined in further experimental projects. All in all, a standard local antibiotic treatment was not able to reach significantly higher cure rates (clinical, bacteriological, cytological) or lower numbers of recurrent mastitis cases compared to the treatment with Pyrogenium® treatment in cows with chronic infections, based on historic milk recording monthly cow somatic cell count data and mastitis history. Regarding clinical cure as one primary outcome, descriptive results showed small differences in cure rates ranging from 54.7% for the reference treatment (CG) to 60.0% for the non-antibiotic treatment. This is in accordance with previous investigations, which reported a probability of clinical cure of approximately 60% for clinical mastitis cases treated with antibiotics, respecting different definitions of clinical cure [12, 13, 14]. Sample size was calculated to give the study sufficient power and to show a difference between test and reference therapy if there was a real difference of at least 15% according to Schukken et al. 2013 [12]. The non-antibiotic treatment showed a numerically almost identical clinical cure rate and no significant differences to the reference treatment.

The other primary outcome variable was the recurrent mastitis rate (R60). The probability of achieving a clinical mastitis recurrence within 60 days after the end of treatment was similar for CG (32.0%) and EG (25.3%). Statistical analysis showed no significant differences between these two treatments. Thus, Pyrogenium® treatment could significantly reduce the use of antibiotics, without influencing the number of recurrent cases of clinical mastitis. Ziesch et al. 2017 found in a similar study in cows with mild to moderate mastitis and a longer lasting high somatic cell count no significant differences due to bacteriological cure and recurrent mastitis between standard antibiotic treatment and a sole therapy with Masti Veyxym®[14].

A study by Mansion-de Vries et al. 2016 could also show a significant reduction in the mean doses of local antibiotics in the examination group, with there being no negative effects on cure rates. The cure rates shown in their study were equal in both the examination and control group [15]. Moreover, 54.5% of their documented milk samples showed no growth or growth of a Gram-negative pathogen, unlike those in our present study (27%). One dominant pathogen on the farms, namely *S. aureus*, particularly affected the cure rates for both the examination and control group. Cases of mastitis caused by *S. aureus* are accompanied with low bacteriological cure rates [6]. The main interests of the farmers are disappearance of clinical signs, a low recurrence rate and a short time of discarding milk [16]. With respect to the primary outcomes, Pyrogenium® seems to show similar results in comparison with the reference group treated with antibiotics. Furthermore, advantageous properties of Pyrogenium® are the zero day withdrawal period for milk and meat, which decreases time of discarding milk, and that it contains no antibiotics, resulting in a reduced risk of residues and improved safety. Another interesting outcome variable for farmers assessing a successful treatment is the course of the cow somatic cell count, because it is used as a measure of milk quality. In this study, cows with persistent high somatic cell counts were chosen and a low probability of bacteriological cure was expected and confirmed.

Conclusion

This concept of non-antibiotic mastitis treatment could show no negative effects on cure rates compared with local antibiotic therapy in the treatment of non-severe clinical mastitis (CM) in cows with longer lasting udder diseases. Having regard to the selection criteria of cows in this study, the findings indicate that sole treatment with Pyrogenium® in non-severe CM cases may constitute an alternative therapy to reduce antibiotics.

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