Udder health effects of an evidence-based mastitis therapy concept in Northwestern Germany

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Abstract

Antibiotic use in dairy farming is a highly discussed issue in society. As a result, the European commission issued guidelines for the prudent use of antimicrobial agents in veterinary medicine in 2015 (EU 2015/C 299/04). Several studies could show effects of selective treatment of clinical mastitis. The present study assesses antibiotic reduction without negative effects on cure rates. The mastitis therapy concept was used on a 950-cow dairy farm in Northwestern Germany from 2016-2017. The cows showing clinical mastitis were assigned to an examination and a control group. The control group (n=71) was given the standard therapy, an intramammary antibiotic. Cows from the examination group (n=69) were treated, based on their individual mastitis history and the result of a rapid on-farm test of a quarter milk sample. The udder health effects such as clinical cure, bacteriological cure, cytological cure, number of recurrent cases of clinical mastitis, the culling rate as well as the withdrawal period and mean doses of antibiotics were compared between the control and examination group. There was a significantly higher chance of a clinical cure in the examination group (p=0.01, examination group: 43.5%, control group: 21.7%). The cure rate for bacteriological cure was 62.5% for the examination group and 66.6% for the control group. As well, 14.5% of cows had a cytological cure in the examination group and 9.9% of cows in the control group, respectively. However, the mean amount of local antibiotics per case was approximately 55% higher in the control group. Thus, this therapy concept could significantly reduce the antibiotic usage for mastitis treatment without there being any negative effects on cure rates.

Key words: dairy cow, clinical mastitis, selective mastitis treatment, antibiotic reduction

Introduction

The antibiotic therapy of clinical mastitis (**CM**) is the most common antibiotic treatment of dairy cows in Europe [1,2]. Due to public concerns concerning the use of antibiotics in live-stock husbandry, alternative therapy approaches are required. Previous studies could show that an antibiotic therapy is not required in all cases of clinical mastitis [3,4,5,6]. Roberson (2003) and Guterbock (1993) claimed that only cases of clinical mastitis caused by Gram-positive pathogens benefit from intramammary antibiotic therapy [30,31]. Cure rates of cases of mastitis caused by Gram-negative pathogens that did not receive antibiotic therapy were even equal to cases treated with antibiotics of Gram-positive cases of mastitis [3]. Therefore, an antibiotic intramammary therapy is not required for all cases of mastitis with a Gram-negative pathogen [7,8,9]. In addition to existing knowledge of the mastitis-causing pathogen, the therapeutic success is also influenced by individual cow factors [3,10,11]. Former mastitis cases in the current lactation and the existence of chronic cases based on the somatic cell count could have negative effects on cure rates [12,10,13]. According to Trevisi et al. (2014), cows with a chronic clinical mastitis should also be excluded from antibiotic therapy [33]. Antibiotics are not indicated in these cases because they did not achieve the expected outcome in animal health [14]. Thus, studying the cow`s history of mastitis before treatment is recommended [13]. Furthermore, studies have shown the positive effects of non-steroidal anti-inflammatory drugs (**NSAID**s) on therapeutic success [15,16]. In conclusion, a therapy concept based on milk test results and data from former mastitis cases should have no lower cure rates with fewer antibiotics than conventional therapy. Therefore, the selective mastitis treatment depending on general condition and signs of inflammation, the cows` former mastitis history and the mastitis-causing pathogen should have no negative effects on the cure rates compared with the standard therapy on the farm - a generally local administration of antibiotic pharmaceuticals. The therapy decisions in the selective mastitis therapy concept were based on the results of a rapid on-farm test. Following the statement of Roberson (2012), it is sufficient to classify the pathogen into one of three categories: Gram-positive bacteria, Gram-negative bacteria or no growth. Only cows with a "Gram-positive" result should be given a local antibiotic therapy. In cases with other results ("Gram-negative" and "no growth") local antibiotic usage could be avoided [17].

The aim of the present study was to use a new selective mastitis treatment concept on a conventional dairy farm. The study was conducted as a randomised controlled trial. The new therapy concept was based on a 12-hour rapid on-farm test in combination with an initial application of a NSAID.

Material and methods

Farm and animals:

The field trial was performed on a conventional dairy farm in Lower

Saxony, Germany, from September 2016 to September 2017. Approximately 950 lactating Holstein-Friesian cows were milked three times a day in a 32-cow rotary milking parlour. The milkers performed a pre-milking of two or three jets of milk before attaching the cluster without any disinfecting pre- or postdip of the teat ends. The average milk yield was 11,311 kg (fat and protein corrected milk, ECM) with an average bulk tank milk somatic cell count below 400,000 cells/mL. Over the entire study period, the clinical mastitis incidence was approximately 55.3% per year. The average new intramammary infection rate (cows with over 100,000 cells/mL in the actual dairy herd improvement of all cows below 100,000 cells/mL in the previous month) within lactation was 31.9%. The most common pathogens on farm in causing clinical mastitis were *Staphylococcus aureus* and *Streptococcus uberis* (data not shown). The cows were kept in a free-stall barn and fed with a total mixed ration depending on their milk yield.

Clinical mastitis cases:

Only cows suffering from CM were assigned to the study. CM was detected by fore-stripping and allocated to one of three categories. Clinical mastitis was defined by the appearance of an abnormal milk character (clots, blood, water →mastitis grade M1), possibly with a swollen and/or heated udder (mastitis grade M2), or, in severe cases, accompanied by additional systemic signs of illness (mastitis grade M3) [18].

Samples:

The milking personnel, trained in detecting and categorising a CM as well as in taking the samples, took aseptical quarter foremilk samples from cows with clinical mastitis, complying with the regulations of the GVA (2009). After cleaning and discarding a few milk jets, teat ends were disinfected with 70% ethanol and a few jets of milk were milked into a test tube with a preserving agent containing boric acid (Ly20) [19]. The samples were used for implementing the rapid on-farm test before being stored at 7°C until being transported to the University of Applied Sciences and Arts in Hannover, Germany twice a week. There, they were examined by researchers unaware of the rapid test results, in accordance with the regulations of the GVA (2009).

Laboratory analysis:

A microbiological analysis of the mastitis milk samples was done following the examination standards as described in a study by Mansion-de Vries et al. (2014) [32]. Laboratory personnel plated ten microlitres of a well-mixed quarter foremilk sample with a sterile loop onto the quadrant of an esculin blood agar plate (Oxoid, Wesel, Germany). The plates were incubated for 48 hours at 37°C under aerobic conditions and examined twice, 24 and 48 hours after inoculation, respectively. The grown colonies were identified by means of their colony morphology, Gram staining, haemolysis patterns and their esculin hydrolysis. Additionally, other biochemical properties such as catalase activity, clumping factor test, Lancefield serotyping, cytochrome oxidase C activity and oxidation-fermentation of glucose were considered for further identification. Deviating from the regulations of the German Veterinary Association, a sample was claimed to be positive for environmental organisms if more than five colonies were identified in the examination (standard operating procedure in the laboratory). As examinations by Smith (1983) indicate, the limit value of ten colony-forming units/0.01mL may be too high regarding coliform pathogens [20]. However, already one colony led to a positive result for cow-associated pathogens like *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Trueperella pyogenes* [21]. If more than two different colony types were detected in one milk sample, the sample was categorised as contaminated. Two different colony types in one sample were referred to as mixed infection.

Experimental procedure:

First of all, based on the farmer's decision, cows that should not be administered any medication on the farm were excluded from the study. These were cows at the end of lactation and those that had an abnormal milk character for a longer period of time without showing systemic signs of illness or only minimal clinical signs of illness (only one clot during pre-milking). Furthermore, cows with a currently high milk yield above herd average in the first half of lactation (subjective perception of the farmer) were also not given any therapy if they had clinical mastitis without systemic signs of illness. The study was performed with two groups: an examination group (**EG**) and a control group (**CG**). Remaining cows with CM were assigned to the two groups based on the cow`s individual neck number on the farm, which had been randomly distributed at the start of the first lactation. All cows with an odd number were assigned to the examination group and all cows with an even number were assigned to the control group. Thus, both groups contained cows with the same characteristics such as days in milk and number of lactations. Cows in the examination group were treated according to the concept in Figure 1. The control group received the standard therapy on the farm, an intramammary application of antibiotics for 2.5 days. There was no determined antimicrobial product. Different intramammary drugs were possible. The study was conducted as a randomized controlled trial. The therapeutic success of the selective mastitis treatment concept in the examination group was compared with the results of the conventional treatment on the farm, the intramammary application of antibiotics in the control group.

Rapid on-farm test:

Every mastitis milk sample from cows belonging to the examination group was tested with a rapid on-farm test on the day the sample was collected. It was noted whether there was a Gram-positive or Gram-negative pathogen present or if there was no pathogen present. The farm personnel implemented and evaluated the tests. Two different rapid tests were used for detecting the mastitis-causing pathogen

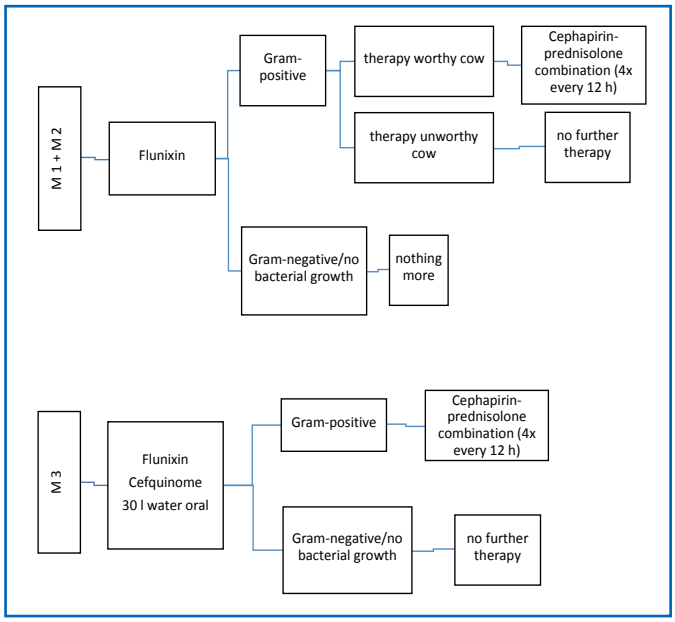

Figure 1: Treatment concept of a selective mastitis therapy concept based on a rapid test considering therapy worthiness and the severity of mastitis (M1 of mastitis = abnormal milk character, M2 = M1 plus swollen and/or heated udder, M3 = M1 or M2 and additional systemic signs of illness).

group. From September 2016 to January 2017, 3M™ Petrifilm™ plates (3M™, Neuss, Germany) were used for detection. The concept was a combination of Rapid Aerobic Count and Rapid Coliform Count which could assign the causative pathogens to Gram-positive, Gram-negative and no bacterial growth [22]. Since January 2017, a newly developed rapid test (MastDecide, QuIdee GmbH, Homberg Ohm, Germany) has been used on the farm. This test was also able to divide the possible test outcomes into the three categories. The test system, consisting of two tubes is based on a colour change of the liquid test medium. The tubes were inoculated with 0.1mL milk, closed, thoroughly mixed and kept in an incubator at 37°C for 12 hours. The liquid test medium only changed from pink to white if bacteria were growing. Thus, no change in colour meant no growth and a colour change in only one tube was interpreted as growth of Gram-negative bacteria. If both test tubes undergo a colour change, it is assumed that Gram-positive pathogens have caused the mastitis [17,23]. Samples from cows in the control group were tested only in the laboratory.

Examination group:

Initially, every cow with clinical mastitis (M1-M3) was administered a cutaneous application of Flunixin (Finadyne® Transdermal, Intervet Deutschland GmbH, Germany) after detection of signs of illness and taking a sample. A one-off application was specified for the recommended therapy. Cows with an M3 additionally received parenteral cefquinome (Cobactan® 2.5%, Intervet Deutschland GmbH, Germany) once a day for two days and were given 30 litres of water orally likewise once a day. Depending on the on-farm test result, only cows with a Gram-positive test result were given an intramammary cephapirin/prednisolone combination (Mastiplan LC®, Intervet Deutschland GmbH, Germany) every 12 hours for two days. Cows with a Gram-negative result or no bacterial growth were not given any local application of antimicrobial products. However, only therapy worthy cows received the intramammary cephapirin/prednisolone combination. Therapy worthiness is defined as an expected therapeutic success. Cows having experienced more than two clinical mastitis cases in the current lactation on the same quarter, and those having more than 700,000 cells/mL milk in the last three dairy herd improvement milk recording tests did not receive a local antibiotic therapy regardless of the on-farm test result. The latter cows have a lower chance of therapeutic success [10]. They only received the Flunixin application once for the actual case as did cows with no bacterial growth or a Gram-negative pathogen in the milk sample. Nevertheless, cows showing systemic signs of illness (M3) were administered parenteral cefquinome even if they were therapy unworthy. The selective mastitis treatment concept for the examination group is shown in Figure 1.

Control group:

After the farmer`s decision, cows in the control group whose milk character was completely lost (thick clots with only a minimal liquid portion) received the standard therapy on the farm based on a local intramammary antibiotic product, independent of a rapid on-farm test. Cows with an M1 and M2 only received a local antimicrobial product. Cows with systemic signs of illness were treated with local antimicrobial products, additionally with a parenteral antimicrobial product and, in cases of subjectively rated poor general condition, with a NSAID. In most cases, a combination of an amoxicillin clavulanic acid with prednisolone (Synulox® LC Plus, Zoetis, Germany) was the first choice of intramammary therapy. It was applied every 12 hours over five treatments. Marbofloxacin (Boflox®, Livisto, Germany) was usually used for parenteral therapy and applied once a day for three days. Alternatively, a one-shot preparation of marbofloxacin (Forcyl®, Vetoquinol, Germany) was applied. If a cow was not clinically cured after the first therapy, the subsequent therapy with intramammary cefquinome (Cobactan® LC, Intervet Deutschland GmbH, Germany) was started. The treatment was independent of individual cow factors like somatic cell count or previous diseases.

Definitions:

The therapeutic success of a treatment is defined by the cure rate which can be differentiated into clinical cure (**CC**), bacteriological cure (**BC**) and cytological cure (**CYC**) [9,24,25,26]. These variables served as a basis for evaluation after treatment of CM. Therefore, two control milk samples were collected by one of the authors 14 (+-3) and 21 (+-3) days after identifying a CM [26,15]. These samples were also examined in the laboratory of the University of Applied Sciences and Arts in Hannover. CC was established by macroscopical examination of the milk, evaluation of the general condition and evaluation of appetite and body temperature. Cows with physiological milk character and an undisturbed general condition were defined as cured. The parameters were evaluated five days after identifying a CM. Macroscopical changes to the milk character at this time indicated that the cow was not clinically cured. A cow was bacteriologically cured when the initial pathogen in the mastitis milk sample could not be detected in both control samples. The CYC was evaluated by means of the somatic cell count which was determined in the laboratory. A somatic cell count below 200,000 cells/mL in both control samples means a cytologically cured cow [3]. All parameters of cure were compared between the examination and the control group. Furthermore, recurrent cases of clinical mastitis, the amount of culling and follow-on treatments (for the actual clinical mastitis case) as well as the mean doses of antibiotics with withdrawal period were evaluated. A recurrent clinical mastitis was defined as renewed CM more than 14 days after the clinical cure.

Number of cows:

The tested hypothesis was that the control group (standard therapy) resulted in a higher bacteriological cure rate (70%) than the examination group (new concept therapy) (45%). Based on a one-sided

Table 1: Amount and distribution of mastitis-causing pathogens in 140 mastitis milk samples from cases of clinical mastitis resulting from a 12-month study on a dairy farm with 950 lactating cows in Lower Saxony, Germany (microbiological culture)

Pathogen	Number of samples	Percentage (%)	Confidence interval (95%)
No growth	37	26.4	(23.7; 29.1)
Streptococcus uberis	36	25.7	(23.0; 28.4)
Prototheca spp.	13	9.3	(7.5; 11.1)
Staphylococcus aureus	13	9.3	(7.5; 11.1)
Contaminated	8	5.7	(4.3; 7.1)
Mixed infections	8	5.7	(4.3; 7.1)
CNS	7	5.0	(3.7; 6.4)
Escherichia coli	7	5.0	(3.7; 6.4)
Streptococcus dysgalactiae	3	2.1	(1.2; 3.0)
Coliform bacteria	3	2.1	(1.2; 3.0)
Enterococcus spp.	2	1.4	(0.7; 2.1)
Bacillus spp.	1	0.7	(0.2; 1.2)
Coryneforme	1	0.7	(0.2; 1.2)
Trueperella pyogenes	1	0.7	(0.2; 1.2)
Total	140		

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Table 3: Cure rates, long-term effects and mean doses of antibiotics with withdrawal period for the examination and control group. The significances are given on the basis of multivariate analysis

	Examination group		Control group		p-value
	amount	percentage	amount	percentage	
Clinical cure	30/69	43.5%	15/69	21.7%	0.013
Bacteriological cure	30/48	62.5%	36/54	66.6%	0.286
Cytological cure	10/69	14.5%	7/71	9.9%	0.959
Recurrent cases of clinical mastitis	9/43	20.9%	2/31	6.5%	0.107
Follow-on treatment	9		13		0.257
Culling	2 cows	2.9%	4 cows	5.6%	0.317
Mean doses of local antibiotics	3.07		4.76		< 0.001
Mean doses of parenteral antibiotics	0.29		0.28		0.822
Withdrawal period	6.11		6.19		0.797

Chi-square test with type I error (α=0.05) and type II error (ß=0.20), a total of 59 animals were required per treatment group. Assuming that approximately 10% of cows drop out of the trial post admission, approximately 65 cows were required per treatment group, in total 130 cows with CM.

Statistics:

The data were collected in Microsoft Access and Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA). SPSS (SPSS 24.0, IBM Corp., Armonk, USA) was used for the statistical calculations. Normally distributed metric data were statistically analyzed 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. BC, CC, and 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/severe), treatment and pathogen type (streptococci, staphylococci, 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, culling rate, follow-on treatments) = Lactation + DIM + mastitis severity + pathogen-group + treatment + pathogen-group x treatment + e

Akaike information criterion was used to determine the model quality.

Table 2: Distribution and composition of cases of clinical mastitis for both test groups and their comparability by p-value

	Examination group	Control group	p-value
Mastitis cases	69	71	
Days in milk*	80 (range: 4-156)	98 (range: 5-191)	0.204
Lactation number†	3 (range: 1-7)	3 (range: 1-8)	0.758
M1	41	45	
M2	22	19	0.617‡
M3	6	7	

* Mean of days in milk

† Mean of lactation number

‡ p-value for all grades of mastitis - to compare the frequency distributions

Results

In total, 140 clinical mastitis cases were included in the evaluation, 69 cases of which belonged to the examination group and 71 cases to the control group. 46 cases were not considered in the evaluation and were disregarded due to false treatment (accidental change of antibiotics or too short treatment), missing control samples or extended therapy, which would distort the evaluation. Based on farmer`s decision, another 382 cases were not enrolled due to high milk yield or minimal clinical symptoms as described earlier. These cows did not get any therapy. However, the two test groups were comparable in terms of days in milk (p= 0.204), number of lactations (p= 0.758) and distribution of mastitis severity (p=0.617). The microbiological results of the mastitis samples are shown in Table 1 and were evaluated for the 140 cases. S*treptococcus uberis* was the most frequent pathogen (25.7%), followed by *Staphylococcus aureus* (9.3%) and *Prototheca spp.* (9.3%). In 26.4% of all cases, no pathogen was found. More than one pathogen (mixed infections) could be isolated in 5.7% of all cases. A detailed composition of control and examination group is shown in Table 2. The bacterial spectrum varied significantly between the two groups due to the uneven distribution of streptococci (more in CG) and staphylococci (more in EG) (p< 0.01, data not shown). Table 3 shows the cure rates allocated to the examination and control group. The bacteriological cure did not differ much between either group (examination group (EG): 62.5%, control group (CG): 66.6%). The difference was not significant neither was the cure rate of the cytological cure (EG: 14.5%, CG: 9.9%). Using the generalised linear mixed model, no significant differences for cure rates except from the clinical cure could be established. The variable "CC" outcome was associated with the mastitis grade (M1-3) (p=0.08). Similarly, the value "BC" was associated with number of lactations (p=0.08) as well as the bacterial group (p=0.115). But, the clinical cure at day 5 after detecting mastitis (EG: 43.5%, CG: 21.7%) was significantly different between the control and examination group (p=0.013). Besides, there was no difference in the amount of follow-on treatments (FOT) (EG: N=10 FOT, CG: N=13 FOT). Nonetheless, more recurrent cases of clinical mastitis were detected in the examination group (EG: 20.9%, CG: 6,5%) with p=0.107, but the culling rate of CG was twice as high as in the examination group (EG: 2.9%, CG: 5.6%; p=0.317). The average withdrawal period in the control group was 6.19 days and 6.11 days in the examination group, respectively (p=0.797). The mean doses of antibiotics for each clinical mastitis were evaluated for local application and parenteral application and significantly differed in the use of local antibiotics between the examination (3.07) and the control group (4.76) (table 3, p<0.001). The null hypothesis

mentioned at the beginning of the paper had to be rejected. Contrary to the assumption, the cure rate of the experimental group is not 25 % lower than that of the control group but even higher for BC (6.2%), CYC (4.6%) and CC (21.8%).

Discussion

A selective mastitis therapy concept was used on one 950-cow dairy farm in Northwestern Germany and applied to cows with clinical mastitis. After excluding cows with high milk yields and those with only minimal clots in their milk, based on the farmer`s decision, cows were enrolled then assigned to the control or to the examination group. The therapy decisions for antibiotic treatment in the examination group were based on a rapid on-farm test, the results of which were available only twelve hours after sampling a cow. Cure rates of both groups were compared to those of other studies. All in all, using a rapid on-farm test in combination with selective mastitis treatment should result in less frequent use of antibiotics without there being any negative effects on cure. As seen in our results, neither the bacteriological nor the cytological or clinical cure in the examination group were inferior to that of the control group (BC: EG: 62.5%, CG: 66.6%; CYC: EG: 14.5%, CG: 9.9%; CC: EG: 43.5%, CG: 21.7%, respectively). There was even a significant higher chance of clinical cure for cows in the examination group compared with cows of the control group. On the other hand, the mean doses of local and parenteral antibiotics in the control group were about 50% higher than in the examination group (EG: 3.07 local antibiotics, 0.29 parenteral antibiotics; CG: 4.76 local and 0.28 parenteral, respectively). Thus, selective treatment could significantly reduce the use of antibiotics. Nevertheless, the withdrawal time was also fairly similar in both the examination and control group. Lago et al. (2011) also found no negative effects on cure rates for strategic treatment of clinical mastitis. They were able to show a decreased withdrawal time of one day in the examination group, which could not be stated in our present study due to a higher withdrawal period of the local antibiotic used in the examination group. 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. Moreover, 54.5% of their documented milk samples showed no growth or growth of a Gram-negative pathogen, unlike those in our present study (20.5%).

The main challenge was the elimination of many cases due to “high milk yield” and minimal clinical signs of mastitis for the evaluation. Due to the fact that these cases had been excluded from evaluation before being assigned to the examination or control group, they did not in any way influence the results. The exclusion, based on the farmer`s choice, was not intended to influence the study`s results but to maintain a high milk yield and to reduce milk losses. As every treatment led to milk losses, the farmer was anxious to treat as few cows as possible. Therefore, all cows with a subjectively perceived high quantity of milk and clinical mastitis only discovered by one clot were not treated in order to minimise the milk losses (382 cases). The udder health effects were not a primary goal for the farm. Thus, this study was conducted under “real life conditions” which did not influence the distribution of the animals to the control or examination group. In addition, further cases were excluded from the evaluation after being assigned to the control or examination group. These were cases due to false treatment, missing control samples or extended therapy. Mostly, the false treatment was characterized by an accidental change of local antibiotics. The cases were not taken into account in order to improve the comparability of the examination and the control group. These exclusions did not introduce a bias in the analysis of the results because the deviations from the inclusion criteria were not purposive but due to the lack of compliance. Under these conditions, the new antibiotic reduced treatment concept showed the same outcomes compared to conventional treatment. Certainly, the results of this study were influenced by the high somatic cell count, corresponding infection pressure and presence of many chronic infections. Due to these circumstances, many cases of clinical mastitis could not be cured. The number of recurrent cases of clinical mastitis was higher in the examination group than in the control group, whereas the culling rate was twice as high in the control group than in the examination group. However, no significance was established. There was no discernible reason for this. Further studies are necessary to show whether this effect is reproducible on farms with a lower somatic cell count and another pathogen spectrum. A larger number of samples and studies on other farms are required to prove these outcomes.

Two of the dominant pathogens on the farm, namely *Staphylococcus aureus* and *Prototheca spp.*, particularly affected the cure rates for both the examination and control group. Cases of mastitis caused by *Staphylococcus aureus* are accompanied with low bacteriological cure rates [27]. Moreover, *Prototheca spp.* could hardly be eliminated from the udder. Culling is the better option [28] and these cases would probably not benefit from an antibiotic therapy [29]. A high number of infections with eukaryotes (yeasts and P*rototheca spp.*) were the major difficulty in this study. The rapid test, made for detecting Gram-positive cocci and Gram-negative bacteria, could not identify eukaryotic cells. Nonetheless, the result was “Gram-positive” in 73.3% of all protothecal cases tested with the Petrifilm test [22]. Clinical mastitis caused by these cells should have been classified as samples with no growth but received antimicrobial products due to the rapid test result. These factors could have led to lower cure rates in both, the control and examination group. However, despite there being a high amount of these pathogens in the samples, antibiotic usage could be reduced in the examination group without there being any negative effects on the cure rates. Furthermore, the bacterial spectrum varied significantly between the control and the examination group. Streptococci were predominantly detected in samples from the control group, whereas staphylococci were mainly isolated in samples from the examination group. However, this effect was excluded from the analytical statistics with concept * bacteriological group.

Nevertheless, the “evidence-based mastitis therapy concept” required greater effort than the standard therapy on the farm. Taking the milk samples, implementing the on-farm rapid test combined with studying every cow’s mastitis history and measuring the temperature were more time-consuming than only administering local antibiotics. Additionally, the evaluation of the on-farm tests after 12 hours and the delayed treatment of a cow after evaluation require more work and defined work planning especially if more than one person is involved in the treatment. The results for the cure and usage of antibiotics in the examination group were affected by the on-farm test results of the rapid test. Thus, the rapid test could have influenced the results. However, the diagnostic certainty was proved for both tests. Compared to a microbiological culture, MastDecide had a sensitivity of 83.6% for Gram-positive pathogens and 72.2% for Gram-negative pathogens. The specificity was 94.1% for Gram-positive pathogens and 83.3% for Gram-negative pathogens, respectively [23]. Petrifilm™ achieved similar results: Sensitivity (specificity) of 93.2% (39.0%) for Gram-positive and 88.9% (97.5%) for Gram-negative pathogens [22]. Therefore,

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both tests could be comparable. Furthermore, the test results could have been influenced by the farm personnel who implemented and evaluated the tests. Thus, professional training in implementing and evaluating the tests as well as taking samples without contamination is necessary and recommended.

This therapeutic concept could lead to a lower use of antibiotics for mastitis treatment without any negative effects on cure. The withdrawal period was not negatively influenced, and the mean doses of antibiotics could be significantly reduced. The economic aspects have to be checked individually for every farm. Additional costs for rapid tests, NSAIDs and operating expenses should be calculated for udder health and reduction in antibiotics. Moreover, this concept depends on the farm personnel and its motivation. The concept could be one approach to reducing the use of antibiotics in mastitis therapy but is no compensation for lacking prevention.

Conclusion

This concept of selective mastitis treatment could show no negative effects on cure rates compared with a standard therapy on the farm with simultaneous reduction of local antibiotics by approximately 50%. Despite the additional expense for work implementing and evaluating the rapid test and cows` mastitis history, the concept could be a step towards reducing the use of antibiotics for mastitis therapy. More studies on other farms with other dominating pathogens and better udder health are necessary to prove this result.

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