Implementation of a targeted mastitis therapy concept using an on-farm rapid test: antimicrobial consumption, cure rates and compliance

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Abstract

Background Aim of the present study was to investigate the implementation of a targeted therapy (tLCT) concept under real-life circumstances, taking both pathogen-related and animal-related factors into account. The reduction of antibiotics without negative effects on cure rates was evaluated as well as the compliance by the farmers.

Methods After analysing the existing conventional therapy (CT) concepts of five farms, the tLCT concept and a novel on-farm test were introduced. Three treatment groups were compared with respect to bacteriological cure (BC), cytological cure (CYC), full cure (FC), new infection rate (NIR), relapse rate and the treatment approach per mastitis case: the CT group, the tLCT group including all clinical mastitis (CM) cases treated according to the concept, and the modified tLCT group (tLCTmod), including the CM cases in which farmers deviated from the concept.

Results Even so farmers deviated from the treatment concept in 506 out of 909 cases; belonging to one of the three treatment groups had no significant impact on BC, CYC, FC, NIR or relapse rate. The antibiotic usage in the tLCT as well as in the tLCTmod group was significantly lower in comparison to the CT group.

Conclusion From this, it can be deduced that farmers will reduce antibiotic doses by implementing a tLCT concept.

Introduction

Bovine mastitis is one of the most costly diseases affecting the dairy industry and is the most common condition affecting dairy cows where antibiotics are used.[1 2] Mastitis is a painful condition which not only impacts animal welfare but also causes great economic losses mainly due to discarded unsaleable milk, reduced milk yield and increased culling rates.[3] Over recent decades, the aims of mastitis treatment have been focused on maximising cure rates and the production of low somatic cell count (SCC) milk, which has led to relatively simple treatment criteria resulting in predominantly blanket antimicrobial treatment (AT) for every cow with clinical signs (bLCT).[4] Although current research has not shown an alarming increase of antimicrobial resistance in mastitis-causing bacteria,[5] there is increasing pressure from the public to reduce antimicrobial usage in dairy production commensurate to other medical sectors.[6] To achieve long-term changes in farmer treatment decisions, new strategies and tools are needed to support and motivate producers.[7] A targeted mastitis therapy concept that considers scientific evidence through the inclusion of both pathogen and animal-related factors, has the potential to be an effective option to reduce antimicrobial doses while keeping cure rates constant, compared with current conventional therapy (CT) concepts.[8]

The basis of a targeted lactating cow therapy (tLCT) concept is to withhold the use of antimicrobial substances where their use conveys no benefit and

Veterinary Record (2020) doi: 10.1136/vr.105674

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Provenance and peer review Not commissioned; externally peer reviewed.

Received August 8, 2019
Revised May 12, 2020
Accepted June 11, 2020

target their use to where they do. Identification of those cases demands knowledge concerning the individual cow as well as the mastitis-causing pathogen. Taking into account factors such as monthly individual cow SCC in dairy herd improvement (DHI) programmes, age, previous mastitis cases and stage of lactation, the probability of cure can be estimated.[9–13] Furthermore, the treatment decision should be based on the causative agent.[8 14 15] Mastitis caused by Gram-negative bacteria, especially coliforms, is characterised by a high self-cure rate and consequently does not necessarily require or justify antimicrobials in mild-to-moderate cases (grade 1 or grade 2).[16–18] Moreover, 30 per cent of samples with clinical mastitis (CM) exhibit culture negative outcomes and AT in those cases should be questioned.[2 14] AT will significantly improve bacteriological cure (BC) rates only in the presence of Gram-positive pathogens, especially streptococci and staphylococci.[17 19] Parenteral antimicrobial therapy should be avoided except in severe mastitis cases with systemic signs (grade 3), where there is a high risk of bacteraemia.[20] In non-severe mastitis cases, intramammary AT achieved higher cure rates with less antibiotic doses being used.[19] As a laboratory examination takes at least 48 hours to identify mastitis-causing pathogens, on-farm rapid tests are a necessary tool for implementing targeted and locally managed AT. No negative effects on cure rates of mild-to-moderate cases (grade 1 or grade 2) have been reported due to a postponed treatment up to 24 hours to wait for on-farm culture (OFC) results.[21 22]

The efficiency of culture-based treatment protocols using tLCT has been proven in previous studies, comparing short-term and long-term outcomes with those bLCT outcomes.[14 22] Antimicrobial use was more than halved in the test groups adopting tLCT without negative impacts on cure rates or udder health key performance indicators, while milk withdrawal times were reduced by three days. Moreover, by including animal-related factors to identify treatment-unworthy cows, a 60 per cent reduction in antimicrobial usage could be achieved when compared with a blanket treatment regimen with no impact on cure rates.[8] Furthermore, a detailed cost analysis confirmed that the tLCT concept saved €40 per case.[8]

In order to achieve a lasting improvement in udder health, a continuous implementation of management measures is required.[23] Ruegg and others[24] identified a general lethargy and failure to motivate producers to pursue long-term goals. However, farmers want to comply with consumer demand for reduced antimicrobial usage and show their commitment and responsibility. Furthermore, the knowledge and awareness of the risk of potential development of antimicrobial resistance are decisive aspects for dairy farmers stimulating them to rethink treatment norms and motivating them to strive actively towards the prudent use of antimicrobials.[25] Nonetheless, different motivators for different farms are likely to be needed to change the behaviour around antimicrobial usage.[7]

The aim of the present study was to investigate the implementation of a more evidence-based mastitis therapy concept under field conditions. For a locally managed OFC approach to achieve tLCT, the novel rapid tube test system mastDecide (Quidee, Homberg, Germany) was used by five farms.

Materials and methods

Farms and previous therapy concepts

The study protocol was approved by the ethics committee of the University of Applied Sciences and Arts Hannover, Germany. The study was initially conducted on eight free-stall dairy farms in Northern Germany from November 2015 to February 2018. However, as three farms did not collect complete data to verify cure rates, only information from the remaining five farms are presented. Farms differed in herd size, farm structure (family business, dairy personnel) and type of production (organic, conventional). These have been selected to represent typical farm structures in Germany. Two farms were family businesses and therefore employed mainly family staff, whereas three farms engaged mainly external employees. All farmers in the study had an agricultural college degree in common with most farmers in Germany. Participating farms had average animal health management, showed an interest in reducing antibiotic usage and were open to alternative mastitis treatment protocols. Only one farm produced organic milk. Average annual milk production was between 9500 and 12,200 kg and bulk tank milk SCCs ranged from 200,000 to 300,000 cells/ml milk. Herd size varied between 175 and 650 milking cows. Between November 2015 and September 2016, the treatment of CM cases was in accordance with the existing CT concept for each of the five study farms. Treatment of all CM cases was observed and recorded to determine antibiotic consumption under the CT concept. Exact treatment data were collected for three farms. For two farms with strict blanket treatment, antibiotic consumption was estimated based on the number of mastitis cases treated. From September 16 to February 18, the novel treatment concept was observed on the five study farms. All CM cases were recorded over the entire duration of the study.

Sampling and mastitis definition

After detection of CM, farmers took a foremilk sample aseptically according to the guidelines of the German Veterinary Association (GVA).[26] Post-treatment quarter samples were taken after 14 (±3) and after 21 (±3) days. All samples were taken in test tubes containing the preserving agent boric acid (Ly20), refrigerated and sent twice a week to the laboratory of the University of Applied Sciences and Arts Hannover, Germany for conventional cytomicrobiological diagnostic examinations according to the GVA,[26] which are based on National Mastitis Council recommendations.[27] Using sterile calibrated loops, 10 µl of each well-mixed milk sample was plated on a quadrant of an aesculin blood agar plate (Thermo Fisher Scientific, Germany). Plates were incubated for at least 48 hours at 37°C under aerobic conditions. Isolates were Gram stained to assist in organism identification. Furthermore, morphology of colonies, aesculin hydrolysis, catalase reactivity (3 per cent H_2O_2; Merck, Germany) and haemolysis patterns were used for identification. Gram-positive and catalase-positive cocci were identified as staphylococci. For differentiation of *Staphylococcus aureus*, clumping factor test (Staph Plus Kit, DiaMondiaL, Vienna, Austria) was performed. Other staphylococci were referred to as non-*aureus* staphylococci (NAS), while Gram-positive catalase-negative cocci were identified as streptococci. For differentiation of aesculin hydrolysing cocci, modified Rambach agar[28] was used. β-d-Galactosidase-positive and aesculin hydrolysing cocci were identified as *Streptococcus uberis*, and aesculin hydrolysing, β-d-galactosidase-negative cocci were identified as enterococci. β-Haemolytic streptococci were characterised by Lancefield serotyping (DiaMondiaL Streptococcal Extraction Kit Sekisui Virotech, Germany). Streptococci from group C were referred to as *Streptococcus dysgalactiae* and from group B as *Streptococcus agalactiae*. Gram-positive, β-haemolytic, catalase-negative irregular rods with V-shaped or Y-shaped configurations were identified as *Trueperella pyogenes*. Gram-positive, catalase-positive, asporogenic colonies on aesculin blood agar were identified as coryneform bacteria. *Bacillus* species form colonies on aesculin blood agar which are catalase-positive and appear as Gram-positive rods forming endospores. Coliform bacteria are Gram-negative, catalase-negative and cytochrome oxidase-negative (Bactident oxidase, Merck, Germany) rods. They metabolise glucose fermentatively (OF basal medium with addition of D (+)-glucose monohydrate, Merck, Germany). Chromocult Coliform Agar (Merck, Germany) was used for differentiation of *Escherichia coli*. After incubation at 37°C for 24 hours, *E coli* forms blue colonies; other coliforms form pink-red colonies. Gram-negative rods showing no mobility during the performance of the oxidative fermentative test were identified as *Klebsiella* species. Gram-negative, catalase-positive and cytochrome oxidase-positive rod-shaped bacteria showing oxidative glucose degradation were identified as *Pseudomonas* species. Yeasts, moulds and *Prototheca* species were differentiated microscopically. Environment-associated, mastitis-causing microorganisms (*S uberis*, *E coli*, NAS, *Klebsiella* species, coliform bacteria, yeasts, *Pseudomonas* species and *Prototheca* species) were recorded as a microbiologically positive result if at least ≥5 cfu/0.01 ml were cultured. National Mastitis Council recommendations are that samples with two identified pathogens are covered by the definition of a mixed infection, whereas samples with more than two pathogens are described as contaminated, except in the event that a colony of a cow-associated microorganism (*S aureus*, *S agalactiae*, *S dysgalactiae* or *T pyogenes*) was found. Somascope Smart (Delta Instruments, The Netherlands) was used to determine the SCC by flow cytometry.

Clinical mastitis severity score

Classification of the mastitis severity score (MS) was done based on the definition by the International Dairy Federation[29]: MS 1 (grade 1) if there was only change in the appearance of milk (colour, viscosity, consistency), MS 2 (grade 2) in the case of additional local clinical signs of the udder (swelling, heat), and MS 3 (grade 3) for cows with general clinical signs (fever, lack of appetite).

Treatment-unworthy cows

Cows with three consecutive high somatic cell scores (≥700,000 SCC/ml) in the previous three monthly DHI data or with more than two CM cases in the current lactation were classified as treatment-unworthy cows, since in this group no higher cure rate can be achieved by antibiotic administration.[30,31]

Targeted lactating cow therapy concept

The tLCT concept is built on three variables, which results in a stepwise approach to the recommended AT: the first variable is the clinical appearance, with the second being animal-related factors and the third being the pathogen-related factors (figure 1). The use of a rapid test on a milk sample taken from the affected quarter immediately after detection of mastitis is necessary for the prompt determination of the pathogen-related factors.

Every cow receives an NSAID treatment immediately after detecting the CM for up to three days.[32,33]

1. Mastitis score: the clinical appearance alone determines whether a systemic treatment should be performed. Only cows with severe mastitis (MS 3) immediately receive systemic antimicrobials and supportive fluids. AT of mild-to-moderate cases (MS 1, MS 2) is delayed while the result of the OFC is pending. A decision concerning the local AT of all cases is made after receiving the result of the OFC (mastDecide approximately 12 hours after diagnosis) (point 3).
2. Treatment worthiness: cows, which are covered by the definition of treatment-unworthy animals receive no local AT as they would not accrue any benefit.
3. Mastitis-causing pathogen: for the remaining treatment-worthy cows, the result of the OFC, and thus the mastitis-causing pathogen, determines if cases receive local AT. Only those with Gram-positive test result receive intramammary antimicrobials, while udder quarters with Gram-negative test result or no verified bacterial growth stay untreated. In cows having an index (first) CM case in their first to third lactation with a Gram-positive test result, an extended local treatment is recommended.[11,34,35]

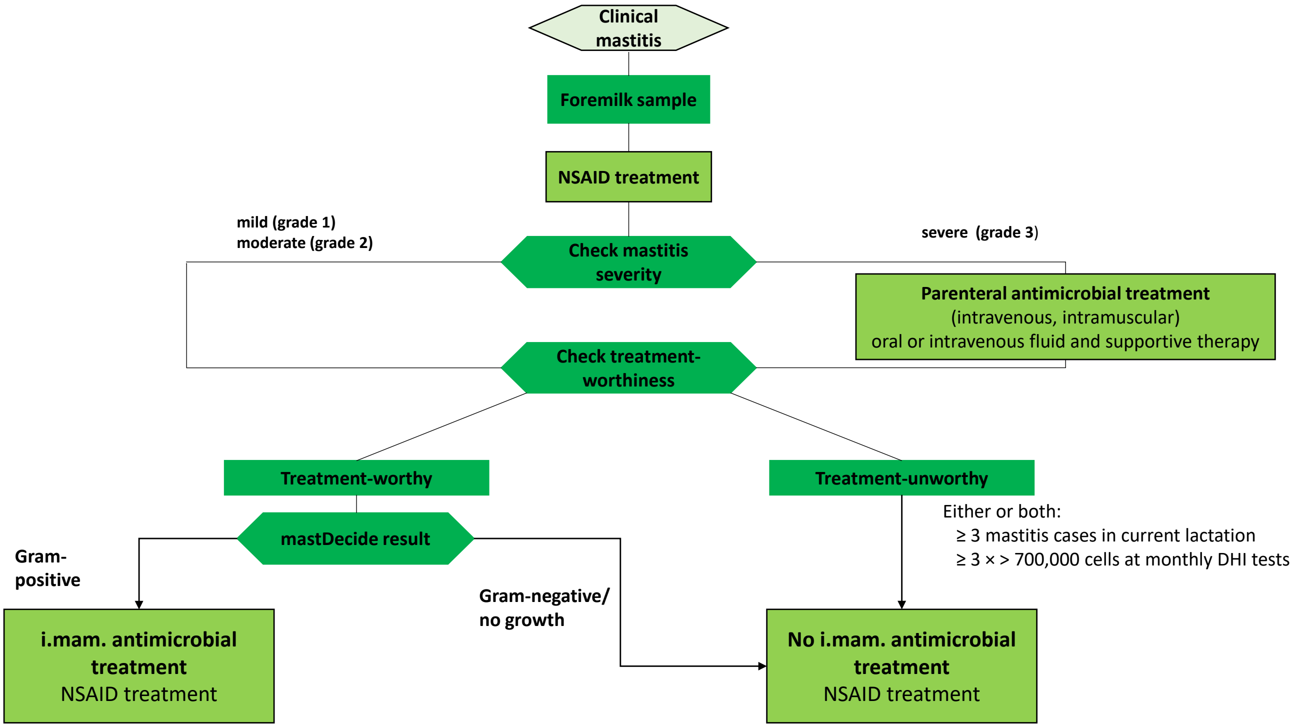

Figure 1 Decision tree. DHI, dairy herd improvement; i.mam., intramammary.

Rapid test phase

From September 2016 to February 2018, the tLCT concept and the novel rapid test mastDecide (Quidee) (https://www.youtube.com/watch?v=uDFFEhv2WoQ) were tried and tested by the dairy employees. mastDecide was performed and evaluated on farm by farmers. After an incubation of 12 hours, an on-farm classification of the mastitis-causing pathogen as Gram-positive, Gram-negative or no bacteriological growth was feasible.[36]

To introduce the tLCT concept and mastDecide, test farms were visited by a veterinarian from the working group. In a joint meeting with all persons involved in mastitis treatment or performing the test, the current state of knowledge on which the therapy concept is based was communicated and the recommended therapy approach was explained. A decision tree (figure 1) along with instructions with illustrations (online supplementary material 1) of the individual procedures to be followed for performing and evaluating mastDecide with the treatment recommendations were handed out to the farms.

Each farm set up a clean working area in a separate room, most commonly an office room to perform the mastDecide test. During the same meeting to introduce the test, each person who would use mastDecide tested it several times under the direction of the attending veterinarian.

Farms filled in a protocol for each mastitis case (online supplementary material 2), containing the MS score, the test result, information about the treatment worthiness of the animal and the treatment given. The protocol was designed to be as easy and as clear as possible and was created with the help of the farmers. It was similar in structure and sequence to the data to be entered and the three variables of the tLCT concept, so that users were guided through its implementation. Therefore, these protocols were not only the basis for the documentation and subsequent evaluation by the working group but also supported the farmers through treatment decisions and filling in their paperwork. The mastDecide test was performed and the results evaluated on farm by the trained dairy staff. Veterinarians from the working group assisted the implementation process and visited farms periodically. In this way, questions arising from the farmers were answered and uncertainties regarding treatment decision were discussed. After completion of the project, a survey of all participating farms was conducted (online supplementary material 3). The survey topics included the practicality of mastDecide and the tLCT concept, difficulties in its implementation and influences on treatment decision making. Information was given in free-text answers. Since all problems and questions were openly discussed with the veterinarians during the study and these were surveyed again with the questionnaire, the questionnaires were not made anonymous. The producers made the medicine choice in consultation with the farm veterinarians. Products were not changed in the study period.

Study design

Objective variables were BC, cytological cure (CYC), full cure (FC), new infection rate (NIR) and relapse rate at a quarter level. The study was intended to show that the introduction of the tLCT concept would keep the target variables constant but lead to a significant reduction in antibiotic usage. In contrast to previous trials, the

Table 1 Descriptive data of composition of the conventional therapy (CT) group, targeted therapy with modifications (tLCTmod) group and targeted therapy (tLCT) group

Parameter	Total N	Treatment group						P value*
		CT group		tLCTmod group		tLCT group		
		n	%	n	%	n	%	
Mastitis case (n)	1392	483		506		403		
DIM								0.11
Heifer	2	1		1				
DIM ≤100	648	209	43.3	251	49.6	188	46.7	
DIM 101–200	370	133	27.5	132	26.1	105	26.1	
DIM ≥201	372	140	29.0	122	24.1	110	27.3	
Parity								0.15
1	285	104	21.5	109	21.5	72	17.9	
2	277	102	21.1	100	19.8	75	18.6	
>2	830	277	57.3	297	58.7	256	63.5	
Mastitis severity score								<0.001
1	737†	206/398	51.8	314/486	64.6	217/400	54.3	
2	412†	154/398	38.7	126/486	25.9	132/400	33.0	
3	135†	38/398	9.5	46/486	9.5	51/400	12.8	
Treatment-unworthy cases‡	161‡	36/482	1.2	76/503	15.1	49/401	12.2	<0.001
Pathogen groups								<0.001
No growth	488	139	28.8	175	34.6	174	43.2	
Coliforms	207	86	17.8	71	14.0	50	12.4	
Streptococci	307	104	21.5	106	20.9	97	24.1	
Staphylococci	169	65	13.5	61	12.1	43	10.7	
Others	221	89	18.4	93	18.4	39	9.7	

*P values based on chi-squared test of each variable by treatment group.
†Of a total of 1284 cases with mastitis severity score information.
‡Number of mastitis cases of animals with three times ≥700,000 SCC DHI or third case in current lactation. Information was available for 1386 cases.
DHI, dairy herd improvement; DIM, days in milk; SCC, somatic cell count.

application of the OFC, the treatment decision and the treatment itself was conducted by the farms in order to examine the feasibility of such an approach under real-life circumstances. Consequently, the study design was a comparison of the objective udder health parameters and antimicrobial doses before and after introducing the tLCT concept to the farms.

A local antimicrobial dose is defined as one udder injector (tube) administered via the streak canal into one mammary quarter. A parenteral dose is defined as one injection of the drug at the dosage and route stated in the summary of product characteristics (SPC).

CM cases during the rapid test phase were classified afterwards into two treatment groups according to the received treatment for statistical analyses. Conditions for a case defined as 'treated as recommended in the tLCT concept' had been treated in one of the following ways: in the case of a treatment-unworthy cow, the patient received NSAID treatment and no local antimicrobials. In the case of a treatment-worthy cow, the patient received NSAID treatment and local AT or not based on the rapid test result. Thus, for a case to be counted as 'treated as recommended', the cow must have received an NSAID, and the treatment worthiness criteria and the OFC result must have been included in the treatment decision. The decision tree had to be followed (figure 1). Conditions for a case defined as 'tLCT with modifications in treatment (tLCTmod)' includes all cases that deviate from 'treated as recommended' (eg, no NSAID was given or local AT was administered despite a Gram-negative test result). This has enabled the analysis of treatment outcomes for cases that fit the tLCT concept as defined by the authors.

This was a non-blinded, non-randomised CM trial with five dairy farms selected as a compliant and convenient group of farms. In the trial, three different treatment concepts (CT concept, tLCTmod concept and tLCT concept) were compared.

The Template for Intervention Description and Replication (TIDieR) reporting guidelines were used to ensure good qualitative research.[37]

Definition of the outcome variables

BC was defined as an absence of the mastitis-causing pathogen in both post-treatment samples. If one post-treatment sample was contaminated, the other one was used to determine the BC. CYC was defined as the SCC of both post-treatment samples being less or equal to 200,000 cells/ml milk. A case was considered FC if there was a BC and a CYC concurrently (in the case of no bacterial finding a CYC was taken to be an FC). A cow was considered to have a NI if the same pathogen was identified in both post-treatment samples, which differed from the mastitis-causing pathogen. A relapse was defined as the detection of a new CM after more than 14 days and up to 90 days after the preceding infection in the same udder quarter.

Statistical analysis

Data for each case were collected in Microsoft Access and Microsoft Excel 2016 (Microsoft Corporation, Washington, Redmond, USA). To test the homogeneity of the data of the three treatment groups, the nominal data (ie, clinical score) were compared as proportions with chi-squared test. The treatment effort in the individual groups was also compared using univariable analysis.

Because the affected quarter within cow was the statistical unit of observation for treatment outcome, clustering was present in the study (quarter in cow, cow in farm). All models contained farm, cow (nested in a herd) and quarter (nested in a cow) as random effects to account for clustering within cow and repeated observations per quarter. BC, CYC, FC, NIR and relapse rate were evaluated using mixed model logistic regression analysis where parity (lactation number; 1, 2, >2), days in milk (DIM; ≤100, 101–200, ≥201), mastitis score (mild, moderate, severe), treatment (CT, tLCTmod or tLCT) and pathogen group (streptococci, staphylococci, coliforms, no growth and other) were included as fixed effects.

The treatment concept was the main variable of interest. SCC cure was categorised according to the cut-off value of 200,000 cells/ml as described earlier. For the statistical analysis, SPSS (V.24.0, IBM, Armonk, New York, USA) was used. The full model was given by:

Logit (BC, CYC, FC, NIR, relapse rate) = parity + DIM + mastitis score + pathogen group + treatment + treatment × pathogen group + treatment × mastitis score + herd (random) + cow (nested within a herd, random) + quarter (nested within a cow, random) + e

A value of $P<0.05$ was considered to be statistically significant.

The multivariable analysis was done using a backward stepwise selection and elimination procedure. After each run, the variable with the highest P value (F-test) was excluded from the model until all variables had $P\leq0.05$. Random effects were not significant in the models but the farm effect was kept as a design variable. The most optimal model was evaluated using the Akaike information criterion (AIC), where an AIC closest to zero was deemed the best model. Model fit was evaluated by checking normality of the residuals.

Results

Description of treatment groups

A total of 1392 mastitis cases were enrolled in the study (483 in the CT group, 506 in the tLCTmod group and 403 cases in the tLCT group) (table 1). The test groups were similar in terms of DIM (P=0.11) and lactation number (P=0.15). For 1284 cases the MS score was determined by the farmers. There were significant differences in the distribution of MS (P<0.001) and number of treatment-unworthy cases (P<0.001). In the tLCTmod group, relatively more MS 1 cases are included compared with the CT and tLCT groups (65.0 per cent in tLCTmod group in comparison to 51.8 per cent in CT group and 54.3 per cent in the tLCT group) (P<0.001). The proportion of treatment-unworthy cases was significantly smaller in the CT group (7.5 per cent) compared with the tLCTmod (15.1 per cent) and tLCT groups (12.2 per cent) (P<0.001). Distribution of pathogen groups varied significantly between treatment groups (P<0.001).

Microbiological findings

In the conventional microbiological investigation, the most frequently detected pathogen was *S uberis* (n=270; 19.4 per cent), followed by *E coli* (n=108; 7.8 per cent) and NAS (n=100; 7.2 per cent). In 35.1 per cent (n=488) of all samples no microbiological growth was found. The detailed distribution of pathogens of the three treatment groups is listed in table 2.

Cure rates and antimicrobial consumption

Table 3 shows the respective cure rates of the individual treatment groups. There were no significant differences between the treatment groups for BC rate, CYC rate, FC rate, NIR and relapse rate. The mean doses for local antibiotic administration differed significantly between the three treatment groups with the highest use in the CT group and the lowest in the tLCT group (P<0.001) (table 3).

In addition, the evaluated mean doses of parenteral antimicrobials showed significant differences with, again, the highest use in the CT group (P<0.001). Conversely, the mean doses of NSAIDs were the highest in the tLCT group, followed by the tLCTmod group and the lowest in the CT group (P<0.001).

Results of mixed regression model

After model building for BC, the remaining explanatory variable was pathogen group (P=0.029), without there being any significance within the group (online supplementary material 4, table 1). For the final model of CYC, again the pathogen group and also DIM met criteria for entry into multivariable model (online supplementary material 4, table 2). Cows with coliforms or streptococci had a significantly worse chance for CYC (coliforms: P=0.008; OR=2.76; 95 per cent CI: 1.31 to 5.80; streptococci: P<0.001; OR=3.48; 95 per cent CI: 1.73 to 6.99). Regarding the period of lactation, model showed that cows had a higher chance of CYC at the beginning of lactation (≤100 DIM: P=0.009; OR=0.55; 95 per cent CI: 0.35 to 0.86). When model fit for FC, the pathogen group was the only remaining explanatory variable (online supplementary material 4, table 3). Animals with mastitis caused by coliforms or streptococci had a significantly worse FC rate than other pathogen groups (coliforms: P=0.004; OR=3.17; 95 per cent CI: 1.45 to 6.93; streptococci: P=0.001; OR=3.39; 95 per cent CI: 1.69 to 6.77). In the model for NIR, the only significant variable was the parity (online supplementary material 4, table 4). The model

Table 2 Detailed microbiological results of conventional therapy (CT) group, targeted therapy with modifications (tLCTmod) group and targeted therapy (tLCT) group based on conventional microbiological diagnostics method (n=1392, mastitic udder quarter milk samples)								
		Treatment group						
		CT group		tLCTmod group		tLCT group		Total
Classification	Results	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n
No growth		139 (28.8)	139 (28.8)	175 (34.7)	175 (34.7)	174 (43.2)	174 (43.2)	488
Gram-positive	*Streptococcus uberis*	91 (18.8)	183 (37.9)	90 (17.8)	172 (34.1)	89 (22.1)	140 (34.7)	270
	Non-*aureus* staphylococci	33 (6.8)		36 (7.1)		31 (7.7)		100
	Staphylococcus aureus	32 (6.6)		25 (5.0)		12 (3.0)		69
	Streptococcus dysgalactiae	11 (2.3)		9 (1.8)		4 (1.0)		24
	Enterococcus species	14 (2.9)		5 (1.0)				19
	Further streptococci	2 (0.4)		6 (1.2)		3 (0.7)		11
	Streptococcus agalactiae			1 (0.2)		1 (0.2)		2
Gram-negative	*Escherichia coli*	36 (7.5)	86 (17.8)	42 (8.3)	71 (13.9)	30 (7.4)	50 (12.4)	108
	Coliform bacteria (except *E coli*)	50 (10.4)		29 (5.5)		20 (5.0)		99
Mixed infections		22 (4.6)	22 (4.6)	12 (2.4)	12 (2.4)	15 (3.7)	15 (3.7)	49
Further pathogens	*Pseudomonas* species	6 (1.2)	24 (5.0)	9 (1.8)	27 (5.3)	2 (0.5)	8 (2)	17
	Coryneforms	10 (2.1)		5 (1.0)				15
	Truperella pyogenes	3 (0.6)		6 (1.2)		1 (0.2)		10
	Bacillus species	1 (0.2)		4 (0.8)		3 (0.7)		8
	Yeasts	3 (0.6)		3 (0.6)		1 (0.2)		7
	Prototheca species	1 (0.2)				1 (0.2)		2
Contaminated		29 (6.0)	29 (6.0)	45 (8.9)	45 (8.9)	12 (3.0)	12 (3.0)	86
Missing samples				4 (0.8)	4 (0.8)	4 (1.0)	4 (1.0)	8
Total		483		506		403		1392

demonstrated that cows in their second parity had significantly less NI in their post-treatment period than cows in their third lactation or higher (P=0.012; OR=2.48; 95 per cent CI: 1.22 to 5.05). The final model for the relapse rate showed no significant predictor at all.

None of the evaluated variables (BC, CYC, FC, NIR, relapse rate) were associated with the variable 'treatment concept'.

Table 3 Descriptive results about cure rates, new infection rate and relapse rate, mean doses of local and parenteral antibiotics and mean doses of NSAID per mastitis case for the conventional therapy (CT) group, targeted therapy with modifications (tLCTmod) group and targeted therapy (tLCT) group			
	Treatment group		
Parameter	CT group	tLCTmod group	tLCT group
Treatment effects			
Bacteriological cure rate	75.3% (137/182)	76.9% (140/182)	78.4% (120/153)
Cytological cure rate	18.7% (54/289)	17.1% (57/333)	17.0% (51/300)
Full cure rate	17.3% (50/289)	16.2% (54/333)	15.0% (45/300)
New infection rate	10.3% 26/252)	11.2% (34/304)	8.7% (25/287)
Relapse rate	8.9% (35/395)	8.7% (42/484)	11.1% (44/398)
Therapy expenditure			
Mean doses of local antibiotics (sd)	5.74* (±4.02)	2.16† (±2.87)	1.54† (±2.49)
Mean doses of parenteral antibiotics (sd)	0.8* (±1,36)	0.3† (±0,91)	0.28† (±0,86)
Mean doses of NSAID treatment (sd)	0.53† (±0.98)	0.68† (±0.89)	1.16* (±0.55)

*†Values with different superscripts are significantly (P<0.05) different based on univariable analysis (post hoc test).

Compliance

During the rapid test phase, farmers did not treat their cows in 506 of 909 cases as recommended in the tLCT concept. Those cases formed the tLCTmod group for statistical analyses. Possible modifications of the treatment concept and case numbers are listed in table 4.

The questionnaire about difficulties in implementation was completed and returned by five producers (online supplementary material 3). The authors have tried to reproduce and summarise the content without being influenced.

Taking a clean sample was seen as the most time-consuming part, whereas the performance and evaluation of mastDecide was easy for the farms. Additional time was mentioned of between 15 and 30 minutes per case. For one farm sampling and treatment with an NSAID were the most time-consuming steps, but overall, they saved time because

Table 4 Possible modifications of the recommended treatment and number of cases during test phase (more than one modification per case is possible)		
Modification of recommended treatment	n	%
No NSAID treatment	258/909*	28.4
Local antibiotics despite no growth or Gram-negative test result†	91/418‡	21.8
No local antibiotics despite Gram-positive test result†	49/207§	23.7
Local antibiotic treatment despite treatment unworthiness	34/125¶	27.2
No usage of mastDecide	170/909*	18.7

*All cases of rapid test phase.
†Treatment-worthy cows during rapid test phase.
‡Cases with negative/Gram-negative test result during rapid test phase.
§Cases with Gram-positive test result during rapid test phase.
¶Cases of treatment-unworthy cows during rapid test phase.

untreated cows did not need to be moved to another milking group to manage the risk of antibiotic residue contamination of bulk tank milk. The additional effort of increased documentation was named during most farm visits. However, the given treatment structure and necessary documentation was seen as a gain. Keeping to the 12-hour rhythm was sometimes a challenge, especially for small family run farms at times of high workload and reports of between one and six weeks were not uncommon to achieve a successful integration into the daily routine.

In the discussions about the interpretation of the test results, some farmers and staff members of the study farms claimed they believed the test was right if their personal suspicion had been confirmed. One saw a reason to doubt the test results if no bacterial growth was shown by mastDecide and another farmer questioned the outcome in cases of severe mastitis with no growth test results.

Farmers described giving more antimicrobials than recommended by the tLCT concept for young cows, high-yielding cows, cows close to birth and recurrent mastitis cases. In these cases, both additional systemic antimicrobials in mild and moderate cases and local antimicrobials despite negative or Gram-negative test results were given. In addition, treatment-unworthy cows with high milk production sometimes received AT. Conversely, in some cases farmers did not treat cows with Gram-positive test results with local antimicrobials if their milk yield did not decrease.

Discussion

The aim of the present study was to prove if a tLCT concept could be successful in everyday life on German dairy farms outside randomised clinical trials. Previous treatment concepts of CM and the treatment outcomes of five German dairy farms were recorded and compared with those after introducing a tLCT concept and the novel OFC mastDecide to the farms. Based on the treatment decision of farmers and farm workers, cases during the rapid test phase were assigned either to a tLCTmod or to a tLCT group. This study was positive controlled, but not randomised as it was seen as a real-life process of implementation. Therefore, the effect of time due to the different treatment phases following one another cannot be removed from the results. Nevertheless, as the results of former randomised studies were already available,[8 38] there was a need for the present study design to take the next step of bringing tLCT onto dairy farms.

Implementation and evaluation of mastDecide was performed autonomously by the trained farm personnel. Supervision of performance did not comply with the objective of the study to observe the outcomes in a realistic everyday life on farm situation. However, this may have influenced the results of the OFC and thus the treatment. In particular, unhygienic sampling technique can falsify the diagnostic outcome due to contaminated (with Gram-positive cocci) samples. This may result in fewer antibiotics being saved. The diagnostic certainty of mastDecide has been confirmed by Leimbach and Krömker.[36] Farms recognised more treatment-unworthy cows during the rapid test phase (7.5 per cent in the CT group v 15.1 per cent in tLCTmod and 12.2 per cent in tLCT group). There may be a number of reasons for this including the introduction of accurate recording of all cases, whereby more animals were documented with their third case of CM the longer the project ran. It is also possible that farmers' newly gained understanding of incurability criteria may also have contributed to their perception of these cases and indicate a behavioural change. The results of this study suggest that implementation of a more scientifically based concept on dairy farms can reduce the amount of antibiotic doses with unaffected cure rates.

In this study, belonging to one of the three treatment groups (CT, tLCTmod and tLCT) had no significant impact on BC, CYC, FC, NIR or relapse rate. From this, it can be deduced that all three treatment concepts with differing labour costs, inputs and drug use led to the same outcomes. The BC rate determined in this study is comparatively high. This might be the effect of the two most frequently detected pathogens, *S uberis* (19.4 per cent) and *E coli* (7.8 per cent), which are both accompanied by high cure rates.[12 18] Several recent studies from Germany have shown that *S uberis* and *E coli* are the most commonly detected mastitis pathogens.[8 13] In a study by Mansion de Vries and others,[8] the most frequently detected pathogen was *E coli* (21.9 per cent), followed by *S uberis* (15.7 per cent). A similar pathogen distribution was also demonstrated in another study in which the mostly cultured pathogen was *S uberis* (34.7 per cent), followed by *E coli* (16.9 per cent).[13] A pathogen-related treatment regimen having no negative effect on cure rates has been shown by Lago and others.[14] An additional inclusion of animal-related factors to the concept, resulting in treatment-unworthy animals receiving no local AT in non-severe cases, was investigated by Mansion de Vries and others[8] and by Kock and others.[38] In both trials, it was possible to show that cure rates in the groups where animal-related factors were included were not inferior to that of the CT groups.

If antibiotic doses are given in accordance with the treatment concept, as much as 73 per cent of locally applied antibiotics could be saved in the tLCT group in comparison to the CT group. In the same way, reductions in parenteral antibiotic use occurs just as dramatically with 65 per cent less doses in the tLCT group compared with the CT group. Despite the treatment recommendations not being fully implemented in the tLCTmod group, about 62 per cent less of both intramammary and parenteral antimicrobial doses were used. In previous trials involving targeted

AT based on OFC results, the intramammary antibiotic consumption was halved[14] and reduced by 70 per cent by Vasquez and others.[22] In a trial with a similar tLCT concept to the present study, Kock and others[38] showed the average consumption of local antibiotics could be significantly reduced, but only by 35 per cent, whereas the mean doses of parenteral antibiotics did not differ to the CT concept group. In a study comparing an evidence-based and conventional approach with mastitis therapy,[8] there was no reduction in mean doses of parenteral antibiotics. Compared with these other studies, the savings on antibiotics in this study are exceptionally high, being the first clinical trial achieving both significantly less intramammary and parenteral antimicrobial usage and unaffected cure rates.

Another aspect that has reduced antibiotic usage is compliance with treatment recommendations according to the manufacturer's specifications (SPC). In practice, farmers tend to prolong treatment if there is no clinical cure after standard therapy.[39] With the introduction of the treatment concept, an extended therapy was recommended according to current state of knowledge only for the first cases in first to third lactation with Gram-positive test result.[35] The awareness that this prolonged therapy is redundant in all other cases has led farmers in this project to treat according to the SPC.

Since the tLCT concept includes an NSAID treatment of all animals with CM, more than twice as many NSAID doses were administered in the tLCT group compared with the CT group. For the tLCTmod group, about 22 per cent more NSAID doses were used in comparison to the CT group. The anti-inflammatory as well as the pain-relieving aspect of NSAID administration are seen as an essential component of the treatment concept to safeguard animal welfare. Furthermore, an NSAID application has shown positive effects on clinical cure, BC and on milk production.[8 33 40] The authors suspected that NSAID administration could possibly prevent tissue damage and shorten the duration of infection.

However, when considering compliance with the treatment recommendations by the farmers, omission of NSAID administration was the most common reason for deviation from the treatment concept. The subsequent division of cases during the rapid test phase into two treatment groups—tLCTmod and tLCT—was based on the treatment decision of the farmers. In more than half of the cases during the rapid test period, farms deviated from the treatment scheme (506 cases in tLCTmod group, 403 cases in the tLCT group) and the common reason for this was that farmers waived the NSAID treatment (28.4 per cent). This result may indicate that farmers underestimate the impact of NSAIDs, even after training. In 27.1 per cent of all non-severe CM cases of treatment-unworthy cows during the rapid test phase, antimicrobials were administered with high milk yield being given as the most common reason for the treatment. In the AT of treatment-worthy animals, 45.5 per cent of the cases during the rapid test phase were treated contrary to the OFC result. This means that cases with a Gram-positive result stayed untreated and those with no bacterial growth and Gram-negative results received an AT. This issue was previously mentioned by Vaarst and others,[4] reporting the same reasons for farmer's treatment decisions as observed in this project. To understand treatment habits and to change them, the commonality of all mentioned reasons is crucial: in this study, farmers believed more in their own assessment of the mastitis case, based on clinical signs and their personal experiences, than to the evidence-based scientific report results. Against the given information and training, farmers still overestimated the impact of antibiotics, in many cases believing them to have a positive influence on the cure rate. The resilience of the survey must be evaluated cautiously due to its brevity and the small number of participants. The results should be seen as an initial opinion and cannot be transferred to the entire dairy farming community. However, the survey gives first insights into the implementation of a targeted treatment concept in real-life situations.

The implementation of a tLCT concept implies extra efforts are required for checking the animal-related factors, taking milk samples, performing mastDecide and intense monitoring of sick cows. However, the additional time required depends on the individual farm structure and staff. Furthermore, the concept must be integrated into the existing management system. Conditions for successful implementation are constant documentation, communication and motivation. The authors' experience in this project has shown that when farms became temporarily negligent in one of the three points, they tend to return to old habits. If, for example, the farm stops recording the CM cases per animal, it is no longer possible to assess the treatment worthiness of the subsequent cases. As a result, even animals that are not worthy of treatment are treated with antibiotics as previously. Therefore, successful implementation must be perceived as an ongoing process in which the current situation must be repeatedly discussed and analysed with the consulting veterinarian in order to maintain or regain motivation.

One conclusion that can be drawn from this study is that farmers will not always fully adhere to the treatment recommendations as provided by the vets or advisors every day or even for every mastitis case. However, despite these deviations from the treatment advice, the usage of antibiotics could also be significantly reduced in the tLCTmod group. Even though the farms have deviated from the tLCT concept, they have changed their treatment patterns compared with the previous phase before the presentation of the novel concept. This could be an effect of the newly gained knowledge. In order to motivate farmers to use antimicrobials prudently, the Dutch government also relied on continuing education.[7] Their RESET mindset model combines five different cues to achieve a long-lasting behaviour change: Rules and regulations, Education, Social pressure, Economics and Tools. As farms took part voluntarily, no compulsory rules and regulations existed in the present study. However, what is consistent is that the farmers in this trial experienced external pressure from public concerns around antimicrobial use in farming, they were educated and trained and received the rapid test as a tool.

A calculation of the mean costs per CM case was conducted by Mansion de Vries and others,[8] comparing the outcomes of a CT concept with those of a tLCT concept and included aspects like costs of drugs, cost of milk discarded during withdrawal periods. Additional costs in the group where animal-related factors were considered included €5.50 for the on-farm test per mastitis case, further equipment and extra time for performing the OFC. Despite these extra costs, it was calculated that an average of €40 was saved per case in the group where animal-related factors were considered. However, two decisive factors must be considered for the evaluation of the economic aspects: the previous therapeutic concept and the pathogen distribution. The conventional therapeutic concept in the study by Mansion de Vries and others provided intramammary AT for each cow, additionally systemic antimicrobials for MS 2 and 3 and sporadically an NSAID treatment. The additional costs in the tLCT group, incurred by performing OFC and more NSAID doses, could be compensated by less discarded milk and a reduction of local and systemic antibiotic doses. The main findings in the study by Mansion de Vries and others[8] were coliform bacteria, which together with cases without bacterial growth, accounted for more than 50 per cent of all cases receiving no local antibiotics in the tLCT group. The application of tLCT on other farms with differing pathogen distributions will result in variations in the proportion of cases receiving no intramammary AT, those justifying AT and those that are treatment-unworthy. For each farm these variations—along with the previous treatment approach—will affect the reduction in antibiotic use as well as the overall economic impact of introducing tLCT.

Due to a probability calculation about long-term cost-effectiveness regarding solely the AT, Down and others[41] conclude that if more than 20 per cent of the mastitis-causing pathogens are Gram-positive, an on-farm culture-based targeted AT is unlikely to be profitable in comparison to blanket treatment. It was estimated that the delay with OFC is at least two milkings, however when using mastDecide as the OFC method this can be reduced to one milking. It was assumed that the on-farm test will be performed for every mastitis case but by considering the animal-related factors before usage of OFC, the number of tests used and associated costs would decrease if farmers pursue economic decision-making. This aspect has not been taken into account in either analysis. Although costs arise from the test itself and its application, if on average local antibiotic doses are saved for each case and the AT cost are similar or sometimes more than the test costs, then the return on investment is at least 1.

During the project period, mastDecide was supplied free to participating farms. Whether conversion to the new tLCT concept is economically worthwhile must be weighed up for each farm and cannot be estimated exactly. Early indications of commercial uptake on farms in the corresponding author's practice and farms in other veterinary practices around the UK shows there is an appetite for the tLCT concept which has delivered reductions in antibiotic use and appears to be sustainable as some herds have been using the concept for close to 12 months. Other benefits of a tLCT concept approach include increased milk sales as milk withholds tend to be shorter without antibiotic therapy, and a reduced risk of bulk tank antibiotic failures as fewer animals are treated with antibiotic, which farmers also consider positive. Although Lam and others[7] counts economic effects to be among the strongest drivers for change, farmers seemed unaware of a reduction of income through milk yield loss following mastitis cases.[42] The Netherlands have therefore decided to impose a penalty for exceeding a certain amount of antimicrobial usage, as farmers react more sensitively to it.[43]

With the results of the current study in mind, the aspect of education and training of farmers and veterinarians could become the main focus of attention in order to ensure long-term success through a change in treatment behaviour. Farmers are willing to reduce the use of antibiotics, not only to cut costs but also to use antibiotics in a prudent way. However, they want to be sure that this will not have a lasting effect on animal welfare or cure rates. Therefore, there is urgent need to inform farmers and veterinarians about evidence-based mastitis treatment but importantly to acknowledge the practices and habits of the farmer to maximise compliance to new protocols.

Conclusion

For many decades, bLCT seemed to be the only option for a high standard of udder health. The current state of knowledge shows that this tends to result in over treating mild, self-curing Gram-negative cases and too little therapy for first cases with staphylococci or streptococci especially for *S aureus* and *S uberis*. Therefore, it is essential that veterinarians and farmers learn about evidence-based results of mastitis therapy such as tLCT. Even though farmers will not adhere to tLCT concepts as strictly as in clinical trials, their usage of antibiotics will decrease significantly while maintaining their level of udder health parameters. The implementation of tLCT concept might take slightly more time in a non-trial situation, however, it does offer a sustainable treatment strategy in a broader perspective. Nevertheless, whatever treatment strategy is used it is essential producers and veterinarians focus constantly on prevention to reduce NIR and to improve udder health at a herd level.

Funding The project was supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

Competing interests None declared.

Patient consent for publication Not required.

Data availability statement Data are available on reasonable request.

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