Farm-specific selective dry cow treatment protocols using a new rapid tube test for identifying mastitis pathogens in dry off milk samples

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Abstract

Blanket dry cow treatment has been recommended and routinely performed on most dairy farms over the years. With increasing findings of antimicrobial resistance, a more targeted approach is now in demand. Selective dry cow treatment (SDCT) aims to only treat treatment-worthy cows with antibiotics. However, implementing SDCT on- farms can be challenging. In this observational study, farm-specific protocols were created and implemented exemplarily on 10 commercial dairy farms in Germany. Regular milk recordings and farm visits were used to find a suitable protocol, taking farmers' concerns about reducing antibiotics into account. Protocols consisted of farm-specific somatic cell count (SCC) thresholds as preselection method and performing a newly developed rapid tube test system for cows below a certain threshold for final decision-making. The tests consisted of a single test tube with pink medium that indicates bacterial growth if the colour turns white after incubation. On-farm tests were performed either on quarter or cow level by farmers or farm staff. Udder health was constantly monitored at herd level through milk recordings (SCC, new infection risk, and cure risk within the dry period), and adaptions of the protocol were made where applicable. Overall, 930 rapid tube tests were performed and compared to cyto-microbiological results either on cow- or on quarter-level, depending on the chosen protocol, and test performance was evaluated for finding treatment-worthy (Gram-positive cocci) infections. Of the 3,093 dry off milk samples from the participating farms that underwent cyto-microbiological diagnostics within the test period, more than 70% showed no treatment-worthy infections, varying between farms with a minimum of 54% and a maximum of 86%. Treatment-worthy infections were found with a sensitivity of 62.34% (CI 54.68; 69.99), and a SP of 73.74% (CI 69.79; 77.69) and an Accuracy of 70.95% (CI 67.41; 74.50) on quarter-level using the on-farm test. The overall udder health, evaluated by dry period new infection risks and cure risks extracted from the farms milk recording data, was not negatively influenced by implementing the SDCT protocols. Some farms even showed an improvement in cure risk (n=6) or new infection risk (n=6). Antimicrobial reduction was estimated at 13.33% over all farms, with less antimicrobials being used when treated on quarter-level. Based on the cyto-microbiological results a potential reduction of over 70% was estimated. It was found that the used on-farm test can help in making more targeted dry cow treatment decisions, while measures to prevent new intramammary infections need to be included in any dry cow treatment protocol.

Keywords: antibiotic treatment, antimicrobial usage; antimicrobial resistance; cure risk; treatment-worthy quarters

Introduction

Antibiotic (AB) dry cow treatment (DCT) has become a common practise in most dairy farms in Germany over the years and still is on many farms, despite of legal requirements to reduce antimicrobial usage (AMU) [1]. Though treating all cows with antimicrobials at dry off (DO), known as blanket dry cow treatment (BDCT), has helped to improve udder health over the last decades, particularly with regard to infectious mastitis pathogens such as *Staphylococcus (S.) aureus* or *Streptococcus agalactiae*, a more targeted treatment approach is needed to reduce the risk of developing antimicrobial resistance. AMU in dairy cows will be monitored more strictly and a reduction of AMU is legally required in Germany [2] and all other member states of the European Union (EU) [3]. While a large amount of AB used on dairy farms in Germany are used for dry cow therapy, there is evidence that not all udder quarters benefit from AB treatment at DO. Hence, there is a high potential for reducing AMU in DCT. However, it is important to find and treat treatment-worthy quarters or -cows at DO so the udder health on those farms is not endangered. This targeted treatment of quarters or cows that are considered to benefit from AB DCT is known as selective dry cow treatment (SDCT). A protective effect of AB to reduce new intramammary infections (IMI) is mainly given at the beginning of the dry period (DP), while the effect is reported to decrease with the ongoing DP [4]. Internal teat sealants (ITS), on the other hand, form a physical barrier,

preventing quarters from new IMI, being a non-AB alternative to protect quarters from new IMI during the DP [5]. In accordance with Regulation (EU) 2019/6, AB must only be used for prophylaxis (in single animals) or metaphylaxis if there is no alternative option to reduce the risk of an infection spreading. Therefore, the risk of new IMI during the DP should be reduced by increasing housing and treatment hygiene [6-9] as well as prophylactic measures being taken to reduce the risk of cows developing milk fever or ketosis around calving [8, 10], while a direct protection can be achieved by using ITS [5]. As a result, only quarters infected at DO need to be treated with AB to increase the cure risk. While previous studies on German dairy farms reported already 46% [11] to over 60% [10, 12] of quarters not showing pathogen growth in the microbiological culture of DO milk samples, a further reduction is possible by only treating IMI caused by certain pathogens. First of all, IMI caused by non-bacterial pathogens, such as yeasts or *Prototheca spp.* can be left untreated, since they are not expected to be affected by AB. Selective treatment protocols based on pathogen-specific selection have already been established in treatment of clinical mastitis (CM) or subclinical mastitis (SCM) [13, 14]. While some studies investigated the effect of treating only CM cases with any positive bacteriological culture, others implemented pathogen-specific selection protocols where in mild to moderate cases only quarters infected by Gram-positive bacteria are treated with an AB, while only in severe cases, CM caused by Gram-negative pathogens are supposed to be treated with AB [13, 14]. As environmental pathogens, Gram-negative bacteria are mainly associated with new IMI. They are described with high self-cure risks, while AB DCT does not seem to increase the cure risk significantly for this pathogen group [11]. Additionally, minor pathogens like coryneform bacteria do not generally cause severe CM and are associated with no or only a slight negative influence on somatic cell count (SCC), milk yield or milk composition [15-18]. Similar observations were found for non-*aureus* staphylococci (NAS), with some studies reporting even protective effects towards IMI with other pathogens, though differences between the NAS species were described [19-21]. Considering the low pathogenicity of these bacteria, a DCT approach with selective treatment of only quarters with an IMI caused by major pathogens at DO, such as streptococci like *Streptococcus uberis, Streptococcus dysgalactiae* and *Streptococcus agalactiae* and *S. aureus*, as recommended in the Norwegian mastitis control programme [22] is possible. However, to only administer AB to cows or quarters that are likely to benefit from the treatment, such as quarters infected with bacteria (to be precise, Gram-positive cocci), these animals or quarters need to be identified at DO. Therefore, different approaches have been studied over the years, including methods based on SCC measurements, like Dairy Herd Improvement tests (DHI) and reports or the California Mastitis Test (CMT), or methods based on bacterial cultures. SCC measurements were investigated at quarter- [23-25] and at cow level [23, 25, 26]. Thresholds of 50,000 – 200,000 cells/mL are described as a good predictor of IMI, with a sensitivity (SE) of 64-86%, dependent on the SCC count threshold used and on whether the samples were taken at cow- or quarter level [23-26]. For cow-level evaluation, accuracy (AC) was better for lower SCC compared to an evaluation at quarter level, probably due to a dilution effect of mixing milk from all quarters of one cow [23, 25]. Overall, with lower SCC thresholds, SE was higher, while the specificity (SP) decreased and vice versa [26]. Using a CMT to identify treatment-worthy quarters also works based on SCC estimations and is a fast way of evaluating the inflammation status of a quarter. SE is described to be > 70% [27, 28], though dependent on what CMT results were used as a cut-off point [27, 29]. Both of these methods work based on the assumption that IMI are associated with elevated SCC, not on direct pathogen detection. However, since not all pathogens cause a significant increase in SCC in the milk, a slight pathogen-specific selection is also expected in this method. Nevertheless, pathogens associated with no or only a slight increase in SCC are mostly minor pathogens and hence might be left untreated without risking CM [26, 30, 31]. Both methods are quite simple ways of evaluating the infection status of a cow or quarter and are linked to low direct costs or workload, which makes them easy to implement on most farms. However, evaluating the infection status based on the SCC gives no evidence of bacterial infection, and hence, further reduction in AMU and more targeted treatment of infected quarters might be possible with methods based on direct bacterial detection. For a more targeted approach, samples could be sent to a laboratory for microbiological diagnostics or investigated via on-farm culture tests (OFT). While laboratory diagnostics deliver quite detailed results (pathogen species or at least pathogen-group) and hence can be useful to gain an impression of which mastitis pathogens are most prevalent on a certain farm, this method is also cost and time intensive. Since for most treatment decisions, less specific results would be sufficient (e.g. treating all IMI caused by bacteria or treating all quarters that show Gram-positive cocci in the milk sample), on-farm diagnostics like rapid tube tests provide a culture-based alternative, where results and treatment decisions can be achieved after 12-24h [32]. Such methods for pathogen detection were investigated and successfully implemented for selective treatment of CM [13, 33, 34]. Likewise, previous studies have investigated the use of an OFT to make SDCT decisions [35-40]. While SDCT trials mainly differentiated between bacterial growth or no growth, studies on selective CM treatment used the OFT to differentiate between Gram-positive (AB treatment) and Gram-negative results as well as no bacterial growth (no treatment) [13].

The objective of this observational study was to implement and assess farm-individual SDCT protocols using a new rapid tube test method and to evaluate this OFT for its ability to detect treatment-worthy bacterial IMI (caused by Gram-positive cocci) at DO in quarter milk samples (QMS) as well as composite milk samples (CMS). The test was expected to be easy to perform and allow on-farm results leading to pathogen-based treatment decisions at quarter- and cow level at DO. Culture-based selection methods can assist decision-making at DO and might encourage farmers to trust in SDCT or support a further reduction in AMU at DO.

Material and Methods

Farms and previous DCT protocols: The study was conducted over a two-year period within the EIP-AGRI (European Innovation Partnership for agricultural productivity and sustainability) selective dry cow treatment project "On-farm test based selective dry cow therapy - reducing the use of antibiotic dry cow treatment in dairy cattle farming". The project aims to develop and implement an innovative way of SDCT, reducing the use of AB at DO to minimise the risk of developing antimicrobial resistance without endangering udder health. Ten conventional dairy farms (farm description with the number 1 till 10) in northern Germany, with a mean average herd size of 203 (SD 133) cows participated in the study. While four farms kept an average herd size of less than 150 cows, five herds had an average size between 150 and 250 cows, farm 8 presented the largest farm with an average of 566 cows throughout the study period. The predominant breed on all farms was German Holstein cows, with two farms keeping single Swiss brown and Swiss Brown x Holstein crossbreds (farms 5 and 6), and farm 9 having individual Simmental cows within the herd. Farms were only eligible if they were already performing milk recordings like DHI on a regular base. While most farms aimed at monthly milk recordings, farm 9 did DHI tests every third month. A preparation period (PP) with farm visits and laboratory diagnostics of milk samples between October and December 2021, as well as the evaluation of the latest milk recordings (May to December 2021) of the farms and developing farm individual SDCT protocols was followed by a test period (TP) from January to December 2022, where those SDCT protocols were put into practice. OFT were performed for cows close to DO

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on an additional farm to increase sample size for the evaluation of test parameters. This farm, however, did not participate in the SDCT trial, hence cows were not treated according to OFT results and findings were not included in the evaluation of the participating farms udder health. Handling of milk samples, instructions for the OFT procedure and evaluation of results were the same for all farms.

Individual farm SDCT protocols: Farmers were questioned about their previous DCT and their requests for an SDCT protocol at the start of the project. Each farm was visited by some of the authors once before creating an SDCT protocol to gather information about farm individual risk factors that might influence the udder health throughout the DP (Table 1). Therefore, farmers were questioned about their milking routine (e.g. teat cleaning and disinfection, milking groups), DO routine (e.g. use of AB and ITS, teat preparation, keeping cows standing after DCT, aimed milk yield at DO), occurrence of fresh cow diseases as well as calving and fresh cow management. During the farm visit, the authors also gathered information about dry cow and fresh cow housing as well as Body Condition Score (BCS) and cleanliness of cows [41] and noted concerns or additional information given by the farmers. Furthermore, QMS were taken by the farmers or farm staff and sent to the laboratory of the University of Applied Sciences and Arts in Hanover, Germany for cyto-microbiological investigation between October and December 2021 to establish pathogen distribution before implementing SDCT. These included DO samples and samples of quarters with CM (Table 2 and 3). Milk recording results made available by the farmers for 2021 and until the end of the project were used to find a suitable SDCT protocol and monitor udder health during the TP. New infection risk (NIR) and cure risk (CR) estimated from milk recording data were evaluated, with the NIR presenting the number of cows with 100,000 cells/mL or less in the last milk recording before DO and over 100,000 cells/mL in the first milk recording after the DP. The CR describes the percentage of cows with more than 100,000 cells/mL in their last milk recording before DO and 100,000 cells/mL or less in their first milk recording after the DP. Results were compared to the mean and top results on German farms, these being 50% and 75% for CR, while top farms reach NIR of less than 15% [42]. If CR were low, a high number of chronically infected and non-curable cows were suspected in the herd. Culling of those was advised to the farmers to prevent them from spreading contagious pathogens, aiming at a number of cows considered as non-curable of below 2%. To account for quarters that were cured but reinfected during the DP, a CR adjusted by the NIR (NIcorrCR) was calculated (CR + NIR*(100-CR)) (Figure 1). If NIR were considered high, prophylactic measures of fresh cow disorders such as ketosis, milk fever and metritis was advised (especially when NIR were higher in cows with >3 lactations compared to younger cows) as well as decreasing infection risks in the cows' environment, which was assessed during the farm visits. On all farms SCC thresholds were used to make primary treatment decisions. While for low CR and high NIR, lower thresholds (higher SE) were advised to start SDCT with, farmers opinions and concerns about using quarter-based SDCT (QSDCT) or cow-based SDCT (CSDCT) and the SCC threshold were also taken into account. The OFT was conducted on all cows below the chosen threshold for further decision making, either at quarter- or cow level. AB DCT was advised for all cows above the chosen SCC threshold and cows or quarters with positive OFT results according to each protocol. For constant NIR below 15%, DCT without ITS was considered. The authors discussed observations during farm visits, results of the investigated milk samples, the latest milk recordings as well as concerns of the farmers about reducing AB at DO in individual online meetings with each farmer in December 2021 and a SDCT protocol was created for each farm (Table 1). SCC, NIR and CR development as well as microbiological results of milk samples were monitored for any changes endangering udder health (increasing NIR, decreasing CR, increasing occurrence of major pathogens) throughout the test period to adapt SDCT protocols if necessary. Farmers were stocked with OFT equipment in early January 2022 to start using the discussed protocols for the next cows that were to be dried off. A second meeting was conducted in June 2022 to gather the farmers' opinions on the ongoing SDCT, discuss possible problems and introduce two newly developed versions of the OFT. Furthermore, a final meeting in January 2023 was held to discuss results and farmers' feedback on the test-based SDCT and the introduced protocols after using them for a year.

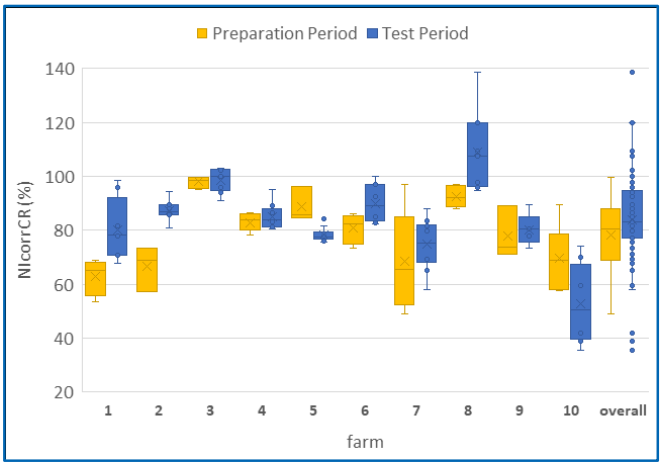

Figure 1: Cure risk corrected by new infections (NIcorrCR) during preparation- and test period overall and for each farm

Sample collection and microbiological analysis: Farmers were stocked with sampling tubes containing a boric acid-based preservative [43] and OFT equipment, including test tubes (at first the same type of test tubes on all farms, different test tubes for QSDCT and CSDCT introduced at the meeting in June 2022), standard disposable 100µl pipettes and a commercial egg-incubator (Incubato® model IN-7DDI; expondo Polska sp. z o.o. sp. k., Poland), before they started with their SDCT protocols. Incubators were checked for temperature fluctuations beforehand and farmers were shown how to use them on site. QMS were taken by the farmers or farm staff of all cows before DO, and, due to each farm's SDCT protocol, investigated with the OFT (Table 1). To get results that best represent the infection status at DO, farmers were advised to take samples and conduct the test right before the planned DO (12-18h). However, timing of sample-taking and testing was due to the farmers decision and ability to include the test in the daily farm routine. All milk samples were sent to the University of Applied Sciences and Arts in Hanover for cyto-microbiological investigation. Microbiological diagnostics were conducted according to the guidelines by the German Veterinary Association using 10µL of milk from each sample [44].

On-farm tests: The OFT used in this study, is a prototype created at the University of Applied Sciences and Arts in Hanover to identify treatment worthy infections at DO. It consists of a single tube with a pink test medium, which indicates bacterial growth if the medium turns white after a 12 h (15 h and 18 h for the in June 2022 introduced versions) incubation period at 37 °C. The farmers had been shown how to perform the OFT by the authors before the SDCT protocols were implemented. A total of 100 µL of each well mixed QMS or CMS was pipetted into a test tube (one tube per milk sample), followed by proper mixing and an incubation at 37 °C. Tests were conducted at quarter- or cow level, depending on each farm's SDCT protocol. For tests at cow level, CMS were created by pipetting equal amounts of milk of each QMS that was taken from one cow into a fresh sampling tube and mixing it. After incubation, the colour of the test medium was evaluated by farmers or farm staff. If any discoloration was seen, the

Table 1: Previous DCT and farm specific SDCT protocols used during the test period

farm	previous DCT (AB used)	farm specific SDCT protocol		Risk factors found on each farm
		Requirement for AM treatment (milk samples used for OFT)	ITS use	
1 AMS	BDCT + ITS (Clox/BFP)	cows with SCC >100.000 cells/mL or cows with at least one OFT positive quarter (qms)	All cows	high proportion of cows unlikely to cure[2] cows lying on slats not cubicles (low cubicle comfort); NIR2>NIR3
2 MP	BDCT + ITS (Clox)	cows with SCC >100.000 cells/mL or OFT positive quarters (qms)	All cows	fresh cows and sick cows milked together history of milk fever problems; NIR2<NIR3
3 MP	BDCT + ITS (BFP)	cows with SCC >100.000 cells/mL or OFT positive cows/quarters[1] (cms/qms[1])	All cows	fresh cows and sick cows milked together; NIR2<NIR3
4 AMS	BDCT + ITS (Clox)	cows with SCC >100.000 cells/mL or OFT positive cows (cms)	All cows	Overcrowded straw bedded part in DC shed; NIR2<NIR3
5 MP	BDCT + ITS (BFP)	cows with SCC >100.000 cells/mL or OFT positive cows (cms)	All cows	fresh cows housed with sick cows, DC housed with in-calf heifers, overcrowding, single cases of retained placenta and *Staphylococcus aureus*, NIR2>NIR3
6 MP	BDCT + ITS (BFP)	cows with SCC >150.000 cells/mL or OFT positive cows (cms)	All cows	DC housed with in-calf heifers, cubicle bedding once a week, cows remain in calving sheds for long periods, NIR2<NIR3
7 AMS	BDCT (Clox)	cows with SCC >100.000 cells/mL or OFT positive quarters (qms)	No ITS	high proportion of cows unlikely to cure[2]; DCT without ITS; *Staphylococcus aureus*; no post dipping after milking; NIR2>NIR3
8 MP	BDCT + ITS (Clox)	cows with SCC >100.000 cells/mL or OFT positive cows/quarters[1] (cms/qms[1])	All cows	fresh cows housed with sick cows; overcrowding; overconditioned DC; long periods with no feed; high NIR; NIR2<NIR3
9 AMS	SDCT + ITS (Clox/BFP)	cows with SCC >200.000 cells/mL or OFT positive quarters (qms)	All cows	high proportion of cows unlikely to cure[2]; fresh cows housed with sick cows; no post-dipping after milking; overcrowding; NIR2<NIR3; high NIR
10 MP	BDCT + ITS (BFP)	cows with SCC > 50.000 cells/mL /cows with SCC >100.000 cells/mL, at least 2 OFT positive quarters or single OFT positive quarters (qms)[1]	All cows	fresh cows housed with sick cows; DC housed with in-calf heifers; overcrowding; short cubicles; cubicle bedding once a week without lime; dirty udders and legs; no pre-dipping before milking; NIR2<NIR3

AB: antibiotic; BDCT: blanket dry cow treatment; BFP: benzylpenicillin-benethamine, framycetin sulfate and penethamathydroiodid containing dry cow tubes; Clox: Cloxacillin containing dry cow tubes; cms: composite milk samples used for the OFT (cow-based testing); DC: dry cow; DCT: dry cow treatmet, ITS: internal teat sealant; NIR: new infection rate; NIR2: NIR for second lactation cows; NIR3: NIR for cows in third or higher lactation; OFT: on-farm test; qms: quarter milk samples used for the OFT (quarter-based testing); SDCT: selective dry cow treatment; AMS: cows milked with an automatic milking system; MP: cows being milked in a milking parlour twice a day; [1]: new protocol started after the second meeting in June 2022; [2]: cows with chronically high SCC

test was considered as "positive" and the quarter or cow was dried off using an AB. When there was no discoloration (test medium remained pink), the test was regarded as "negative" and the cow or quarter was considered to be dried off without AB treatment. OFT results were compared to the microbiological findings in the laboratory. Therefore, the outcomes of the laboratory microbiological analysis were classified according to the probability of the OFT to detect treatment-worthy IMI (Gram-positive cocci) as negative (no bacterial growth, non-bacterial pathogens, bacteria except Gram-positive cocci) or positive (Gram-positive cocci). Mixed (two different colony types) samples were regarded as positive if at least one of the pathogens was Gram-positive cocci. Samples with more than two different colony types were regarded as contaminated. If one of the quarter milk samples of a cow was regarded as contaminated, the associated composite milk sample was regarded as contaminated as well.

Data analysis: Data were collected and edited in Microsoft Excel® (2016). Estimated means of CR and NIR were calculated using SAS studio® (2002-2020, SAS Institute Inc., Cary, NC, USA). SE, SP, negative predictive value (NPV), positive predictive value (PPV) as well as Accuracy (AC) were determined with a 95% confidence interval for each test and incubation period using WinEpiscope (http://www.winepi.net/ 22.06.2023).

Results

Farms and previous DCT protocols: The 305d milk production overall was 10,828kg of energy corrected milk (SD 755). Cows were generally dried off abruptly on all farms, while all, except farm 7, used ITS (containing bismuth subnitrate) on all cows for DO (Table 1). Only farm 9 did not practise BDCT before the trial, to sometimes treat cows with tylosin prior to DO depending on the result given by the AMS and his own assessment. Most farms milked fresh cows in the same group as sick cows or had no special milking order. Dry cow housing varied between farms from straw or slatted free-stall housing to having them out in the field during summer. The number and results of laboratory diagnostics, including clinical mastitis, from the PP are shown in Tables 2 and 3. The main risk factors found for each farm are shown in table 1. All farmers were willing to establish SDCT on their farms, guided by the authors, taking their current udder health status into account.

Farm-specific SDCT protocols: Overall, 409 DO milk samples from nine farms were sent to the laboratory for cyto-microbiological investigation before implementing test-based SDCT protocols on the farms. Of these

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samples, 78 (19.07%) were considered contaminated (more than two different bacteria), though varying between farms (Table 2). Furthermore, in 136 (41.09%) samples, no bacterial growth was found.

Positive microbiological results consisted mainly of environmental pathogens, with coryneforms and NAS being the most common finding (Table 3). Two samples showed growth of *S. aureus* as part of a mixed infection. However, contagious pathogen findings were rare and only appeared in a few DO samples on each farm. Additionally, 93 milk samples of cows with mastitis were investigated (Table 2). However, only seven farms sent in milk samples from cows with mastitis. Farm 10 had not submitted any milk samples prior to the test period.

An overall mean NIR of 24.88% (SD 10.39) was reported in milk recordings before the start of the TP. Most farms showed higher NIR in 3rd and higher lactations than in 2nd lactation cows (farms 2, 3, 4, 6, 8, 9, 10). Though no farmer reported any problems with ketosis and only isolated incidents of milk fever or retained placenta were seen on some farms, subclinical cases

Table 2: Number of milk samples investigated in the laboratory during the preparation period

Farm	Dry off milk samples		Mastitis milk samples	
	Overall n	Contaminated n (%)	Overall n	Contaminated n (%)
Overall	409	78 (19.07)	93	13 (13.98)
1	35	4 (11.43)	16	2 (12.50)
2	64	9 (14.06)	16	5 (31.25)
3	80	14 (17.5)	0	0 (0)
4	55	15 (27.27)	2	0 (0)
5	66	14 (21.21)	6	0 (0)
6	31	11 (35.48)	5	4 (80.00)
7	12	0 (0)	36	2 (5.56)
8	11	6 (54.55)	0	0 (0)
9	55	5 (9.09)	12	0 (0)
10	0	0 (0)	0	0 (0)

were expected to still increase the risk of NI and prophylactic measures were advised. Additionally, increasing DCT and calving hygiene were advised if NIR were considered high, especially on those farms that showed higher NIR in 2nd lactation cows compared to older cows (n=3). The mean DP CR was 58.29% (SD 11.67), varying between 44 and 66% on most farms with three farms remaining constant > 69%. Risk factors found most often during the farm visits included overcrowded sheds and dry and/or calving cows and fresh cows grouped with sick cows (mastitis/lame). Six farmers reported bedding dry cow cubicles or straw sheds at least once a day (farms 2, 4, 5, 7, 8, 9), while two reported bedding once per week (farms 3 and 6) and two bedded them "when needed**".** Udder- and leg hygiene scores for dry cows were estimated at scores 1-2 on most farms, with single cases of higher scores (farms 5, 6), while dry cows on farm 10 were estimated with an overall score of 3 [39]. Cows were kept standing for at least 30 min after the DO procedure on eight of the farms (farms 1, 2, 3, 5, 6, 7, 8, 10).

Farm individual SDCT protocols are shown in Table 1. While QMS were taken for cyto-microbiological investigation in the laboratory, the OFT was either conducted on each QMS of a cow (quarter-based testing) or CMS were created from the taken QMS for cow-based testing, according to each farm's SDCT protocol (Table 1). While 4 farms (farm 1, 2, 7, 9) started with quarter-based testing in January 2022, 5 farms (farm 3, 4, 5, 6, 8) rather tested on CMS. On farm 1 the farmer tested at quarter level, but preferred treating all quarters if one of the quarters tested positive in the OFT. Only one farm had NIR constantly below 15% achieved by using ITS, so using ITS was advised on all farms. However, on farm 7 the farmer decided against ITS due to concerns about introducing. Farm 10 had not submitted milk samples before the start of the trial and presented CR constantly below 55%, with decreasing numbers of 'healthy cows', while reporting being short-staffed. Therefore, treating all cows with an SCC above 50,000 cells/mL with AB DC tubes, while cows below that level were dried off with ITS only was chosen as an SDCT approach to start with.

Table 3: Microbiological findings of milk samples that were sent to the laboratory, excluding contaminated samples

Microbiological findings	Preparation Period		Test Period
	Dry off n (%)	Mastitis n (%)	Dry off n (%)
Overall	331 (100)	80 (100)	2059 (100)
no growth	136 (41.09)	27 (33.75)	1145 (55.61)
Gram-positive cocci	82 (24.70)	22 (27.50)	607 (29.48)
NAS[1]	72 (21.75)	19 (23.75)	447 (21.71)
Streptococcus spp.	6 (1.81)	3 (3.75)	56 (2.72)
Staphylococcus aureus	4 (1.21)	5 (6.25)	10 (0.49)
Enterococcus spp.			18 (0.87)
Lactococcus spp.			27 (1.31)
Aerococcus spp.			1 (0.05)
coryneforms	73 (22.05)	6 (7.50)	196 (9.52)
other Gram-positive bacteria	3 (0.91)		22 (1.07)
Gram-negative bacteria	8 (2.42)	10 (12.50)	86 (4.17)
coliforme bacteria	8 (2.42)		77 (3.74)
Pseudomonas spp.			7 (0.34)
other Gram-negatives			2 (0.10)
mixed infections	29 (8.76)	10 (12.50)	49 (2.38)
mixed infections with Gram-positive cocci	24 (7.55)		48 (2.33)
Non bacterial			2 (0.10)

[1]non-aureus staphylococci

This was considered a very safe SDCT protocol with a low risk of leaving infected cows untreated, while giving the farmer time to improve udder health on the farm before introducing the rapid tube test. The choice of AB and sealer depended on each farm's individual preference. Hence, farmers could stick to their previously used products.

Adapting the test performance and SDCT: In the first meeting after implementing the rapid tube test in June 2022, some uncertainty regarding test results were reported, as discoloration was sometimes only mild or only seen after the incubation period was over, though overall the test was well accepted. Test function and procedure were summarized, and previous results were presented to all participating farmers and questions concerning SDCT and the protocols were addressed and discussed throughout the meeting. In general, the farmers were confident about carrying out the test and treatment according to test results and reported that the protocol was only not followed in isolated cases. With the OFT being further developed in the laboratory throughout the project, evaluating SE and SP for different versions of the OFT on QMS and CMS, an improved version was introduced to the farmers in June 2022. Two different test tubes were used for quarter- and cow-based testing. The incubation times for the new tubes were, based on their highest SE and SP in the laboratory evaluation, 15h for CSDCT and 18h for QSDCT, while except for

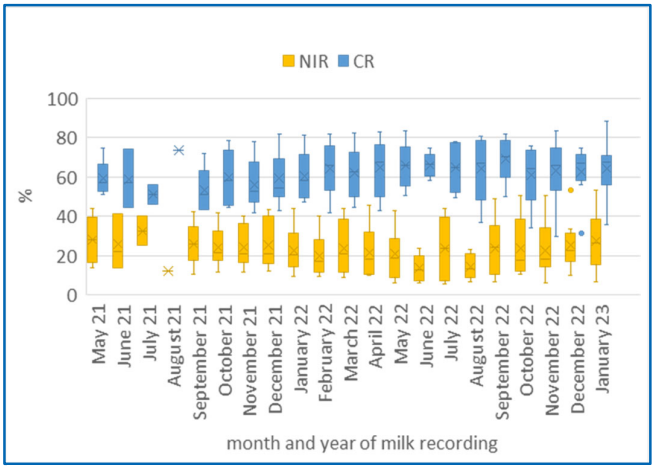

Figure 2: Development of cure risk (CR) and new infection risk (NIR) throughout the study period preparation- and test period)

the prolonged incubation period, handling of samples and performing the test stayed the same. Farm 3 and 8 decided to change from CSDCT with testing CMS to QSDCT with using QMS for the OFT throughout the test period. The reasons they gave for this change were the lesser workload for more precise results when testing on quarter level, since QMS were taken either way to be sent off to the laboratory, while CMS had to be taken additionally or mixed from the QMS. Farm 10 started using the OFT for QSDCT following a farm visit after the meeting. Since the farm still had a staff shortage and udder health issues according to milk recording data, the new SCC threshold was set at 100,000 cells/mL, while all cows above that level were treated with AB DC tubes. Cows with an SCC threshold below 100,000 cells/mL in their last milk recording were tested with the rapid tube test at quarter level and treated according to the results, though cows were treated at cow level if more than two quarters were tested positive in the OFT.

Evaluation of udder health throughout the test period: Milk recording data were evaluated until January 2023 to assess the TP from January to December 2022, where the OFT was performed and milk samples were sent to the laboratory. Overall, 3,093 (2,059 non-contaminated) milk samples were sent in by the farmers for cyto-microbiological investigation within the TP, showing 55.61% culture negative results and 29.48% Gram-positive cocci (Table 3). DHI data were submitted for 1,035 cows dried off during the project, of which 857 were dried off during the TP. The mean milk yields of cows at the last milk recording prior to DO was 27.76Kg (SD 1.82) during the PP and 28.22Kg (SD 1.40) during the TP. However, milk yields at DO that were reported by the farmers when sending in the milk samples showed a mean of 20.59 kg (SD 6.61) for October to December 2021 and 21.89 kg (SD 6.13) for the TP in 2022. For farms 1, 3, 4, 5, 6, 7, 8 and 9 the mean milk yield was slightly higher throughout the TP. The progression of NIR overall is shown in Figure 2, where the mean NIR before the TP was 24.88% (SD 10.39). However, NIR varied between the farms and milk recordings. The overall mean NIR within the test period was 22.19% (SD 12.82). While on four farms (1, 3, 6, 8) overall a slight increase in DP NIR was seen, six farms showed an overall lower mean NIR throughout the test period (2, 4, 5, 7, 9, 10). Eight of the farms stayed below a NIR of 28% throughout most of the study period, while farms 8 and 9 stayed above that value for the entire time. On both farms, keeping fresh cows together with sick cows on a straw bed as well as an overcrowded shed was considered a high risk for new infections. Furthermore, farm 9 did not use teat dips after milking. Farm 8 had reported some issues with milk fever, while long breaks between silage feeds were documented by the authors during the first farm visit. More frequent feeding and measures for milk fever prophylaxis had been advised to the farmer. CR were higher throughout the TP, with a mean of 64.44% (SD 12.82) compared to a mean of 58.29 (SD 11.67) in the PP (Figure 2). CR on most farms stayed above 50%, with only farm 10 presenting CR constantly below 50%, while large variations were seen in NIR and hence NIcorrCR. On that farm, a second herd visit was conducted and persistently high SCC cows were identified for culling. The farmer took advice on improving milking and housing hygiene by using teat wipes and changing from bedding cubicles with sawdust to chopped straw with lime for cows of 5-200 days in milk, and culled persistent infected cows. However, the shed was still overcrowded, fresh cows were housed together with sick cows on deep straw bedding, and dry cows were kept in the field. This might partially explain the increase in NIR in winter after a drop in summer, which had also been seen in the previous year.

Test performance: A total of 909 OFT were performed, including 785 (86.36%) tests at quarter level and 124 (13.64%) tests conducted at cow level. For 22 (2.42%) conducted OFT, that farmers had send in results, there was no sample for laboratory evaluation due to milk samples leaking or being lost during postage, hence those tests were not used for the comparison between OFT result and laboratory diagnostic. Further, test results of milk samples being considered contaminated in the laboratory evaluation were not used for the comparison, leading to an exclusion of another 185 (20.35%) OFT results, of which one was also reported to be inconclusive by the farmer. Finally, 702 test results were left for the evaluation, including 630 (89.74%) tests at quarter level and 72 (10.26%) tests at cow level. Testing at quarter level showed an SE of 62.34% (CI 54.68;69.99) and an SP of 73.74% (CI 69.79; 77.69) to identify Gram-positive cocci with an accuracy of 70.95% (CI 67.41; 74.50), while a positive predictive value (PPV) of 43.44% (CI 36.90; 49.97) and a negative predictive value (NPV) of 85.82% (82.44; 89.20) were calculated. At cow level, test results showed an SE of 55.81% (CI 40.97; 70.66), an SP of 55.17% (CI 37.07; 73.27) and an AC of 55.56% (44.08; 67.03). A total of 545 (59.96%) OFT showed no discoloration (493 quarter- and 52 cow-based tests), indicating that no treatment worthy bacteria were found. However, only 444 (409 QMS and 35 CMS) of these OFT results were included in the test evaluation. When considering only *Streptococcus spp., S. aureus* and *Enterococcus spp*. as treatment-worthy infections instead of all Gram-positive cocci, the SE for testing at quarter level increased to 78.05% (CI 65.38; 90.72), with an SP of 67.91% (CI 64.14; 71.68), an AC of 68.57% (CI 64.95; 72.20) and an NPV of 97.80% (CI 96.38; 99.22), while PPV decreased to 14.48% (CI 9.84; 19.12).

Farmer compliance and AM reduction: Overall, the farmers expressed no general concerns about leaving animals untreated based on the OFT results. However, some deviations from the SDCT protocols discussed were seen when comparing the test results and reported treatment with the protocols. These included AB treatment of cows or quarters that were negative in the OFT, deviations from the specified incubation time, testing cows that were above the set SCC limit or not testing cows below the selected SCC threshold. Farmers reported that deviations from the treatment protocol were due to concerns about udder health in single cows with several cases of mastitis, episodes of high SCC during the lactation or a high milk yield at DO. Furthermore, some farmers reported a high positive rate and that cows tested at quarter level were seldom completely negative. Therefore, in some cases, they treated all quarters with AB even though only some showed positive test results, due to concerns about leaving false negative quarters untreated. However, some farms reported using 50% less AB already, while less reduction in testing CMS was seen compared to QMS. Starting SDCT with a low AB reduction rate seemed to reduce concerns about endangering udder health. Nevertheless, some farmers reported preferring a simpler cow side test with instant results for future SDCT. This was especially the case for farmers with AMS, who expressed a much heavier workload at DO, since cows had to be caught twice (to take a sample

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and for DCT) instead of only once for the DCT.

Discussion

Udder health throughout the test period: As previous research shows, most IMI found *post partum* (*pp*) are new IMI [10, 47, 48]. Whereas less than 11% of IMI were found to persist throughout the DP in previous studies, Nitz et al. reported that over 89% of udder infections found within the 3rd week *pp* were in fact new IMI occurring after calving [10, 47]. Therefore, identifying risk factors for new IMI throughout the dry period, particularly in the calving sheds and fresh cow environment play an important role in improving DP outcomes. CR calculated from SCC values from milk recording data cannot differentiate between persistent infections and reinfections. In fact, the longer the time between calving and first milk recording, the less precise the result is for the actual DP outcome. Calculating the adjusted CR will compensate for some of the influence by new infections and give a more precise estimate of the actual CR, though still influenced by the time of the milk recording. In this study, udder health seemed to be stable throughout the test period and even improved, with an overall 6.15% (SD 12.36) higher CR and a 2.69% (SD 11.97) lower NIR. This meant an overall decrease in NIR by 11%, while the CR increased by 11% over all farms. The NIcorrCR were higher within the test period compared to before, though showing a slightly smaller increase compared to CR (Figure 1). This can be explained by the influence of new infections on CR given by milk recording data. Hence, some of the increased CR was probably due to the reduction in NIR achieved by improving hygiene at DO and fresh cow environment. Only farm 5 and 10 showed an overall lower NIcorrCR during the TP. On farm 10 however this resulted mostly from lower NIR and a slightly lower CR during the first half of the TP, while an increase was seen in the second half, after the second farm visit and the farmer taking measures to reduce risk of infection. Further, even though the farm had not sent milk samples within the PP to compare pathogen distribution during the TP with, the pathogen distribution showed about 67% culture negative samples which is above the mean over all farms, while a lower number of samples with Gram-positive cocci (25%) was detected. Changes on farm 5 were minor and mainly presented in a decrease in NIR at the start of the TP. General advice to increase CR and lower NIR was discussed with all farmers in meetings and certain farm individual risk factors were pointed out to each farmer before starting the SDCT protocols (Table 1). Reducing infection risk by separating fresh cows from sick cows, reducing cow numbers in the shed, prophylaxis of fresh cow sicknesses and culling cows unlikely to cure (chronically high SCC) was pointed out most often. However, decisions and taking action was left up to each farmer in the end. Variations in CR and NIR between the farms in this trial were expected, resulting from different farm setups, staff situations, milking routines and herd sizes. Further, the length of time between milk recordings may have influenced the reported NIR and CR and caused some differences between farms. Especially for farm 9, who routinely performed milk recordings every third month, the reported high NIR and low CR are likely influenced by the prolonged time between DO/calving and the related DHI sampling for some cows. With overall 53.60% of all samples (TP and PP) showing no growth in the laboratory, and coryneforms and NAS being the predominant pathogens, the bacterial findings are comparable to previous studies on DCT [8, 10-12, 48, 49]. The number of culture-negative samples was even higher throughout the TP (55.61%) than in the PP (41.09%). For farm 2, 3, 6, 7 and 8 more than 20 % more samples showed no growth during the TP than the PP, while only for farm 4 the percentage of culture negative samples was lower than in the PP. The overall distribution of pathogens appeared similar before and within the TP. While NAS stayed on the same levels, coryneforms were found less throughout the TP. This is reflected in the lower amount of Gram-positive non cocci of 10.64% in the TP compared to 24.17% in the PP. Gram-negative bacteria were overall rare in the investigated samples (Table 3). Overall, 29.48% of the samples taken within the test period showed Gram-positive cocci, hence bacteria considered as treatment worthy, however differences in the distribution on the various farms were seen. The reduction in environmental pathogens found in the milk samples is likely due to farmers taking measures to reduce NIR at sample taking as well as during milking (wearing gloves, teat dipping or spraying, dry cleaning of teats) and increasing housing hygiene. Nevertheless, with overall 31.70% of the sent in DO milk samples being considered as contaminated (more than two different pathogens), the number of contaminated samples was regarded as high. This means 31.70% of the samples could not be used for a treatment decision based on culture results. As for the OFT, 20.68% of the results were excluded from the evaluation due to contamination found in the laboratory results. Hence, correct aseptic sample taking is key for treatment decisions based on microbiological analysis and can have great impact on the test results. However, in this trial using the samples that were used for the OFT for the laboratory diagnostics as well may have increased the number of contaminations found in the laboratory. Farmers had been instructed in proper handling of samples and performing the OFT during the first meeting and were informed if high rates of contaminated milk samples were detected in the laboratory, though no practical training was conducted with farmers and staff before implementing the SDCT protocols. However, contaminated samples are unlikely to result in leaving infected quarters untreated, hence no risk for udder health is expected, but can increase the amount of unnecessarily treated quarters. An aseptic sample taking and a clean environment for performing the test will improve the accuracy of the test outcome by reducing the risk of positive results caused by contamination. Though sample taking and test procedure were discussed with the farmers, demonstrating and training aseptic sample taking and test performance on the farm could be considered for future use of the OFT if loads of contaminated samples are expected.

Test performance and AMU: Tests on QMS overall performed better than tests on CMS. This finding is likely to have been caused due to a dilution effect by mixing an infected quarter with non-infected ones and due to a higher risk of contamination when mixing the QMS to get a CMS. Additionally, since less farmers were using CSDCT and there was only one sample per cow used for the evaluation, the sample size was much lower in CMS. Though the OFT were overall regarded as easy to perform, farmers reported difficulties in sticking to an exact time frame when reading the test results. Incubation times reported for the performed tests varied between 12 and 20h. For the first test with an incubation period of 12h, no shorter incubation times were reported, however 14-18h was reported in a few cases. For the test introduced in June with an aimed incubation time of 18h for QMS, some deviation towards shorter incubation periods (15-16h) were reported, while only in one case an incubation time of 20h was reported. However, the incubation period actually used was not always reported by the farmers. In these cases, if no alteration was documented, an incubation time according to the given instructions for each test was assumed. Alteration from the aimed incubation period may have influenced test performance results. Since samples with slow growing bacteria or very low bacterial counts might turn positive the longer the sample is incubated, longer incubation times were associated with higher numbers of positive samples and lower false negative results, hence a higher SE. Vice versa, SP is expected to be lower the longer a sample is incubated. This can allow adaption of the test to single farms in future SDCT protocols by choosing, for example, longer incubation times to start SDCT on farms with low CR and high NIR until udder health has improved. Additionally, the longer incubation times reported for the first OFT version could partly explain why some farmers registered low numbers of negative OFT results. Overall, regarding QMS, 62% of the tested quarters infected with Gram-positive

cocci were found by the rapid tube test, showing that including the test in decision-making can help increase the SE while reducing unnecessarily treated quarters compared to using lower SCC alone. Regarding test performance on QMS, the rapid tube test was comparable to previously tested methods of decision-making. While SP was similar to the on-farm culture systems investigated for SDCT by Cameron et al. and Patel et al. [36, 50], SE results were lower. In both studies DO samples with no signs of clinical mastitis were used to compare the OFT results to the laboratory findings. However, other than in this trial, sampling and testing procedures were performed by study technicians. Other methods of decision-making investigated in previous studies, such as SCC thresholds either from the last milk recording (SE 63-78.6%; SP 63.0%-80.5%) or CMT showed comparable test results [23, 24, 27]. Though, SE and SP vary according to the threshold used, the pathogen distribution and what pathogens are regarded as treatment worthy. SCC thresholds found as sensible to identify IMI have been reported as being between 100,000 cells/mL [23, 24] and 200,000 cells/mL [23, 25, 51] in the past. Variations can be due to prevalent pathogens, since minor pathogens are less likely to induce intense SCC responses [23, 52, 53]. Furthermore, thresholds for QMS differ from thresholds on CMS, with better test results being achieved for lower thresholds for CMS compared to QMS, probably due to a dilution effect in CMS [23, 25]. Sanford et al. reported higher SE for CMT at cow level (70-86%), while SP were overall lower (46-48%) [27]. Nevertheless, reported test characteristics show large differences among previous studies, depending on the chosen cut-off points, the definition of pathogen positive results (all pathogens vs. major pathogens) and whether interpretation was conducted at cow- or quarter level [27, 29, 52]. While higher SCC (DHI as well as CMT) thresholds are accompanied by a lower SE, SP is higher and vice versa [24]. However, caution is advised when comparing tests and performance in different studies. Samples tested with the OFT in this study were taken from cows that were preselected by the individual SCC threshold on each farm, while in other studies all cows or quarters were tested with the investigated method or different eligibility criteria were in place. Milk yields were overall high in regard to previous studies showing an increased risk of IMI in early lactation for cows with high milk yields at DO [54-56]. In this study, milk yield was not included in the implemented SDCT protocols, though decisions by the farmers were based on milk yield in individual cases. Reducing the energy uptake in the feed of high yielding cows or gradual milk cessation can reduce milk yield prior to DO and hence reduces the risk of new IMI [57, 58]. Therefore, milk yield thresholds could be included in DCT protocols, for example by separating cows in different feed or milk cessation groups before DO based on their milk yield. Vilar and Rajala-Schultz suggest a target milk yield of 15kg/d or less to be reached by DO to reduce the risk of milk leakage, to help with involution and form a keratin plug and therefore reduce the risk of new IMI [57]. However, while high milk yields prior to DO increase the NIR [57, 59], an influence on the CR is not expected [11, 12, 60]. Therefore, milk yield thresholds such as 15kg/d to separate cows in different feed or milk cessation groups as part of SDCT protocols can help reduce NIR, though should not directly affect the choice between AB and non-AB DCT in individual cows. In this study, some farmers had also diverged from the given treatment protocols in single cows due to cases of CM in the previous lactation. Also not included in the SDCT protocols created within this research, a history of mastitis cases within the previous lactation was associated with a higher risk of CM in the early lactation in previous studies [39, 61, 62]. Furthermore, Gundelach et al. found a reduced chance of cure for quarters showing lumps or nodular indurations on udder palpation [12]. While Vasquez et al. [62] and Rowe et al. [39] included the history of mastitis cases in their algorithm based SDCT protocol, other studies investigating an OFT for SDCT did not include mastitis cases in their SDCT protocol [29, 37, 38]. However, chronically infected cows with constant high SCC and/or repeated mastitis treatments and low chances of cure should rather be considered for culling and could be left untreated [22, 24, 61]. Overall, there are many different methods, and reducing NIR should be a main goal for any SDCT, while combining different methods for decision-making can increase SE, AC and NPV to find treatment-worthy cows and quarters [24]. Performing the OFT leads to an increased workload compared to using DHI data alone or cow side tests with instant cow side results like CMT, especially on farms with AMS where cows have to be caught twice for the test and the actual DCT. However, no cow side test with instant results based on bacterial growth has been developed yet. So far, only few studies have investigated a culture-based OFT approach for SDCT, with incubation times ranging from 12h-48h [36, 38, 50] while the one tested in this trial had the shortest incubation period and could deliver results closest to actual dry off. The overall reduction in AMU compared to BDCT on the ten participating farms was estimated at 13.33%, taking into account that the treatment protocol was not always followed. Regarding QMS only, an actual reduction of 22.21% was estimated for the farms testing on quarter level throughout the whole TP, while a potential reduction of AMU of 26.51% was calculated assuming treatment was given strictly according to the test result. Due to the different approaches used on the farms, variations in AM reductions were expected. With some farms using a 'safer approach' with higher SE, the risk of leaving infected quarters untreated is reduced, while overall more quarters are treated, leading to a lesser reduction in AMU. Also, testing CMS leads to more non-infected quarters being treated with AB, which results in a lower decrease in AMU compared to QMS. Regarding the results of the cyto-microbiological investigation in this study, AMU could be reduced by 70.52%, when treated on quarter-level. Keeping in mind that AB treatment at DO is only shown to cure existing bacterial infections, while new infections can be prevented with management, hygiene and ITS, AMU can already be reduced by 55.61% when treating non-infected quarters with ITS only. Similar pathogen distributions were found in previous studies in Germany, with over 60% bacteriological negative samples at DO [10-12]. Further reduction is possible by pathogen-specific treatment. Infections with Gram-negative bacteria are generally due to new IMI, while high self-cure rates are reported with no significant increase in chance of cure when treated with AB [10, 11, 48]. Other studies have found that even in mild to moderate CM cases, quarters infected with Gram-negative bacteria can be left untreated or treated with anti-inflammatories alone, while CM and SCM caused by Gram-positive bacteria required AB treatment [14]. Additionally, some Gram-positive bacteria like coryneforms or some NAS were found to have a low pathogenicity [18, 63] and could be regarded as not treatment worthy. Excluding bacteria with low pathogenicity and high self-cure rates, treatment can be further narrowed down to only treating Gram-positive cocci, excluding some NAS. In our analysis, a further 14.91% of the samples showed pathogens not considered as treatment worthy. However, NAS species were not identified in this study; hence, all NAS were regarded as treatment worthy. Therefore, a further reduction would be possible, considering that some NAS as well are associated with low pathogenicity, and AB treatment is not associated with increased CR. With 21.71% of DO milk samples showing NAS in this study, in theory, a reduction in used AB at DO of more than 90% could be possible by only treating according to microbiological results. Nonetheless, this is considering none of the quarters with NAS as treatment worthy, while more research is needed regarding the pathogenicity of NAS. Also, pathogen distribution varies between farms, and hence does the possible AM reduction.

Farmers' compliance and SDCT protocols: Bearing in mind that most quarters showed no pathogen findings at DO and even at least one third of the cultured pathogens were not treatment worthy, these findings might encourage farmers to leave cows without AB treatment at DO. While the

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correct identification of treatment-worthy cows or quarters at the time of DO is important for implementing SDCT on farms, the costs, workload and complexity of the test also have a great impact on the practicability of the chosen method. Tests should be low in costs, easy and quick to perform and interpret as well as enable to identify treatment-worthy cows or quarters at the day of DO. While SCC thresholds based on milk recording data represent the method with the least workload and costs on farms that already conduct milk recordings regularly, the results are limited to CSDCT, and the time gap between the last recording and DO might be several weeks. While cyto-microbiological diagnostics in a laboratory are the gold standard to identify pathogens in milk samples and allow pathogen-specific QSDCT, costs and time between sample taking and results are high compared to an OFT. Decisions on CMT on the other hand can be made at the day of DO with minimal workload and low test costs, though no pathogen-specific results are possible. Cyto-microbiological diagnostics, CMT and OFTs enable farmers to perform QSDCT, which allows a greater reduction in AMU than treatment at cow level. However, in Germany AB tubes certified for DCT are in fact not licensed for single quarter treatment, which sets the boundary higher for QSDCT from a legal point of view. To motivate farmers and veterinary practitioners to perform QSDCT and encourage further reduction, more research is needed to have tubes licensed for QSDCT available. OFTs like the one evaluated here allow evidence-based decision-making on the farm within 24h before DO. The test procedure and evaluation are simple and easy to learn and allow a culture-based SDCT approach, though increasing the workload of drying off cows compared to using SCC or CMT only. Combining different methods, e.g. preselecting by SCC thresholds and only testing certain cows, like in this study, allows to further adapt SDCT protocols to a farm, with increasing SE and reducing AMU, while keeping costs and workload to a minimum. Further research is needed to estimate workload and costs of using the OFT on farms for SDCT. Nevertheless, most quarters are not infected with treatment-worthy bacteria at DO, and reducing the NIR has a much greater impact on the DP outcome than the correct identification of infected quarters alone. Previous studies have focused on identifying risk factors that increase the risk of new IMI, including milking, drying off and calving hygiene [8], cleanliness of cows as well as housing hygiene [6, 8, 59, 64]. Additionally, individual cow factors like high number of lactation [35, 42, 65], milk yield at DO [54-56, 59, 65-67], high teat end scores [67] and fresh cow sicknesses [8, 68] have been reported to increase the NIR. While clean application of DC tubes and calving hygiene play the most important role for high NIR in 2nd lactation cows, preventing fresh cow sicknesses like milk fever and ketosis should also be targeted if NIR are high in older cows. The importance of new IMI and approaches to reduce NIR need to be communicated to farmers and farm staff as well as different SDCT methods. Our findings show that in general SDCT can be implemented on every farm without undue risks and even improving udder health by identifying risks for new IMI and constantly monitoring udder health. Farmers and veterinary practitioners should focus on reducing NIR and start with a protocol with high SE instead of aiming for the highest possible reduction in AMU. Especially farms with presence of contagious pathogens like *Strep. agalactiae* or general udder health problems need to focus on identifying the infected quarters (a high SE) and reducing risk factors on the farm. However, they are, keeping that in mind, able to perform SDCT without a deterioration in udder health. Low numbers of IMI *pp* through preventing new IMI and low numbers of untreated infected quarters as well as understanding the reasons behind the chosen methods will help build up farmers' compliance and trust in drying off cows selectively. Farmers' concerns, current udder health and the ability to use certain methods on a farm need to be considered when implementing SDCT on individual farms. Veterinary practitioners should guide farmers when creating a suitable protocol for their farm and making possible adaptions to improve the outcome as well as help them with their concerns.

Conclusion

In this research, farm individual SDCT protocols were developed by reviewing farm- and herd-specific factors influencing DP outcome. Protocols were created according to each farm's udder health and adapted throughout the project. SCC thresholds and a newly developed culture-based OFT system were used in decision-making. SDCT was found to have no adverse effect on udder health as long as udder health was constantly monitored and SDCT adapted if needed. Identifying and reducing risks of new IMI as well as identifying persistent infections play a major role in improving udder health throughout the DP. With most quarters at DO showing no pathogen growth or being infected with not treatment worthy bacteria, a reduction of AMU of over 70% could be possible by identifying treatment-worthy quarters alone. An OFT like the one evaluated in this study can help to increase SE of chosen protocols and enable farmers to further reduce AMU by making culture-based decisions at quarter level.

Disclosure of conflicts of interest

The authors declare no potential conflicts of interest.

Compliance with Ethical Standards

All applicable guidelines for the care and use of animals were followed. The study was approved by the Animal Welfare Committee of Hannover University of Applied Sciences and Arts, Germany. An application for a license for animal testing was not required by the local government due to the observational character of the study. The study met the International Guiding Principles for Biomedical Research Involving Animals (1985).

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