Efficacy of targeted therapy of environmental mastitis using on-farm culturing in small dairy herds

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Abstract

The aim of the study was to evaluate treatment protocols for improvement of clinical and bacteriological cure rate of non-severe clinical mastitis (CM) and selective dry cow therapy (SDCT). Mastitis management based on on-farm commercial culture system MicroMast™ and data analysis were implemented into two dairy herds. Quarters with evidence of Gram-positive agents were treated using benzyl penicillin or amoxicillin and/or by amoxicillin with clavulanic acid. In herd A, 31 quarters of non-severe CM were recorded. Of them, *Streptococcus uberis* was isolated in 16 (51.6%), non-aureus staphylococci (NAS) in 8 (25.8%), *E. coli* in 5 (16.1%) and no growth in 2 (6.5%) cases. Bacteriological cure was observed in 24/29 (82.8%) quarters. Antimicrobial consumption was reduced from 3.56 to 2.33 doses per case. Under the SDCT protocol, 28 quarters (13.2%) of 53 cows dried-off were included, achieving a reduction by 86.8% on the quarter level. In herd B, 23 quarters of non-severe CM were recorded. Of them, *S. uberis* and *Escherichia coli* were identified equally in 8 (34.8%) samples and NAS in 2 (8.7%) samples and with no growth in 5 (21.7%) cases. Bacteriological cure was achieved in 17/18 quarters (94.4%). Antimicrobial consumption was reduced from 4.45 to 1.83 doses per case. Only 5 (1.7%) quarters of 72 cows were included for SDCT with reduction in consumption of antimicrobials by 98.3% on the quarter level. In summary, innovated treatment protocols based on results of on-farm culture enabled a significant reduction of antimicrobial consumption and improvement of bacteriological cure rate in conditions of practise.

Clinical, subclinical, SDCT, antimicrobial consumption, cure

Mastitis is a persistent health problem in dairy cattle, not only negatively impacting the health of dairy cows, but also interfering with animal welfare. The disease causes significant economic losses through direct costs of diagnosis, treatment and elimination of wasted milk from the human consumption but also by indirect costs because of decrease of milk production and the premature culling of dairy cows. Mastitis is an infectious disease of mostly bacterial origin that occurs after an intramammary infection (IMI), which triggers an immune response in the mammary gland with many changes in the milk and varying degrees of pathological damage to the glandular tissue.

Rapid recognition of mastitis and its causes by clinical and laboratory methods at an early stage of inflammation is crucial for making the diagnosis and designing an effective treatment to achieve complete recovery (Leimbach and Krömker 2018; Mansion-de Vries et al. 2014; Ruegg 2017). Antimicrobial drugs of various groups were commonly used for the treatment of non-severe clinical mastitis (CM) of dairy cows. A major problem in the treatment of mastitis has been the threat of the emergence and spread of antimicrobial resistance (Pol and Ruegg 2007).

Therefore, there is a continuing need to address the issue of improving the effectiveness of IMI treatment within programmes of rational usage and reduction of antimicrobial consumption at the herd level. Increasing treatment efficacy is not possible without further

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innovation of diagnostic procedures aimed at detecting the bacterial agent by culture or molecular techniques, e.g. polymerase chain reaction (Dohoo et al. 2011), which is subsequently subject to pathogen-specific treatment. A significant improvement in this direction has been achieved by the simplified on-farm culturing of milk samples (Lago et al. 2011a; Lago et al. 2011b), which has made it possible to obtain preliminary results 18–24 h after sample collection. As part of this trend, a number of other on-farm culture systems for the isolation of major pathogens of environmental and contagious mastitis in dairy cattle have been described (Prasek et al. 2010; Royster et al. 2014; Viora et al. 2014; Ganda et al. 2016).

A valuable benefit associated with implementation of these procedures was the ability to use the culture results obtained on the farm immediately to make the treatment decision for CM with or without the use of antimicrobials (Mansion-de Vries et al. 2016; Lago et al. 2016) and also for the management of selective dry cow therapy (SDCT) during the dry period (Patel et al. 2017; Rowe et al. 2020; McDougall et al. 2021). This will fulfil the general requirement for evidence-based therapy in veterinary practice, including appropriate use of antimicrobials. However, this concept requires increased involvement of veterinarians in development of a mastitis pathogen-specific treatment protocol adapted for herd conditions.

In our study, we focused on the possibility of using on-farm cultivation in two small dairy herds in order to implement innovate treatment protocols for clinical and subclinical mastitis and SDCT during drying-off with the choice of narrow spectrum penicillins (benzyl penicillin) or broader spectrum penicillins (amoxicillin; and also, amoxicillin in combination with clavulanic acid). The aim was to evaluate clinical and bacteriological cure rates in clinical and subclinical mastitis and SDCT and consumption of antimicrobials in herd.

Materials and Methods

The study was conducted in two small dairy herds. Herd A was characterized by a closed turnover of Holstein dairy cows with a milk yield of 7,340 kg per cow/lactation at the start of the study. The average size of herd A was 59 cows during the study. The dairy cows were housed in free stall open sheds with deep bedding. The stable was divided into two areas. The feeding area which was cleaned twice a day and the resting area with daily straw bedding.

Herd B also had a closed turnover of Czech Fleckvieh dairy cows with a milk yield of 7,155 kg milk per cow/lactation. The number of cows was an average of 106 cows during the study. The dairy cows were housed in free stall brick barn using deep straw bedding similar to herd A. A covered outdoor feeding area was cleaned once a day and fresh straw bedding was added in the indoor resting area daily.

Mastitis management in both herds (A and B) during the period before trial was based on antimicrobial treatment of all CM cases (grade 1 and 2) until the disappearance of clinical symptoms while subclinical mastitis was not managed at all. For drying-off blanket dry cow therapy (BDCT) was used constantly. The study was conducted on farm A from November 2021 to the end of November 2022 (13 months) and on farm B from April 2021 to the end of April 2022 (13 months).

On-farm cultivation

Milk sampling was carried out according to standard procedures. Only quarter milk samples were enrolled during the study from a mammary gland affected by non-severe CM (stage 1 and 2) (Pinzón-Sánchez et al. 2011) or suspected subclinical mastitis. Quarter samples were collected from cows in the high-risk category according to the algorithm used at the drying-off with SDCT. The effectiveness of antimicrobial, or spontaneous treatment for bacteriological cure was checked in the quarter sample.

All samples were inoculated onto 3 different agar media on one plate in a commercial MicroMast™ (Světlá nad Sázavou, Czech Republic) set for on-farm culture (Prasek et al. 2010) immediately after collecting. After inoculation, agar plates were incubated directly on the farm aerobically at 36–37 °C for 18–24 h. After that, the bacterial growth was evaluated and interpreted by a trained (non-microbiologist) person according to the manufacturer's instructions. If no colony appeared on any of the media used, the result was interpreted as "no growth". Gram-positive bacteria were differentiated for staphylococci and streptococci based on morphology and catalase positive and negative reaction, respectively. Identification of *Staphylococcus aureus* was based on coagulase positive reaction using commercial test (Staphaurex™ Latex Agglutination Test, Thermo Fisher Scientific Inc). All isolates showing negative coagulase results in test were recorded as non-aureus staphylococci (NAS). Isolates of *Streptococcus uberis* were identified according to morphology of colonies on blood agar

and esculin positive reaction. In case of growth suspected as being *Trueperella pyogenes* or *Corynebacterium* spp., incubation was prolonged for further 20–24 h. Identification of Gram-negative bacteria was focused on *Escherichia coli* and *Klebsiella* spp. In the case of presence of two different species on the plate, the result was interpreted as a mixed infection.

Treatment protocols for CM of grade 1 (mild) and 2 (moderate)

The use of antimicrobials was specified only for non-severe CM with evidence of Gram-positive agents. For intramammary administration in herd A, amoxicillin with clavulanic acid was used in a total of 3 doses (at 12 h intervals) for grade 1 CM (abnormal milk). The treatment of grade 2 CM (abnormal milk and quarter affected) was based on parallel administration of amoxicillin with clavulanic acid intramammary and parenteral injection of amoxicillin for 4 days (2 doses at a 48-h interval).

In herd B, the treatment of grade 1 CM was based on intramammary administration of benzyl penicillin in a total of 3 doses (at 24-h intervals). Grade 2 CM was treated contemporarily with benzyl penicillin for 3 days administered intramammarily and parenterally.

On day 7 after antimicrobial treatment, further clinical examination and a control culture of the milk sample from the treated quarter were performed to evaluate the clinical and bacteriological cure rate.

In contrast, quarters affected by (grade 1 and 2) CM with a detection of Gram-negative bacteria in the sample, as well as quarters with no growth result were not treated by antimicrobials (Lago et al. 2011a). Non-steroid antiphlogistic drugs (NSAIDs) and/or anti-inflammatory ointment administered on the udder skin were recommended to manage the pain and welfare of cows in all CM cases.

Treatment protocols for subclinical mastitis

The protocols were based on the evaluation of the type of pathogen confirmed by on-farm culturing, duration of the infection, days in milk, history of mastitis cases and milk production. In herd A, amoxicillin with clavulanic acid were used for intramammary administration in a total of 3 doses (at 12-h intervals). In herd B, benzyl penicillin was administered intramammarily in a total of 3 doses (at 24 h intervals).

Bacteriological cure was checked by cultivation of the second batch of milk samples. The cure rate in no growth samples was evaluated using somatic cell counts (SCC) according to dairy herd improvement (DHI) data (SCC < 150,000 cells/ml in heifers and SCC < 200,000 cells/ml in cows) (De Vliegher et al. 2018).

Treatment protocols for selective dry cow therapy (SDCT)

In both herds, antimicrobial treatment was supposed to be applied only to cows classified into high-risk category, i.e. in cases when *S. aureus, Streptococcus agalactiae, S. uberis* (including mixed infections), *Corynebacterium* spp. and persistent NAS infection was proven in the quarter. Cows of both herds A and B belonging to high-risk category were treated by intramammary drugs containing a combination of benzyl penicillin, nafcillin, and dihydrostreptomycin registered for dry cow therapy. All quarters of all cows received an internal teat sealant at drying-off to prevent new intramammary infection. Cows with culture evidence of another pathogen or no growth result were classified into low-risk category without the use of antimicrobial treatment. Cows with either a DHI test SCC < 100,000 cells/ml during the last 3 month of lactation and no evidence of clinical mastitis case during the last 30 days of lactation were also included in the low-risk category. To evaluate the basic udder health indicators both in the herd and on the individual cow level, regular DHI testing data and farm records were analysed using the Keno™-M software (Ghent University, Ghent, Belgium) regularly every month.

Some isolates were sent to the Institute of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, Veterinary University Brno and/or the State Veterinary Institute Jihlava to confirm identification by MALDI-TOF and determine antimicrobial susceptibility.

Results

Clinical mastitis

In herd A, a total of 31 cases of CM were recorded, of which 23 (74.2%) were mild and 8 (25.8%) moderate cases. Of the 31 samples examined, the causative organism was confirmed by culture in 29 (93.5%) cases. The most frequent *S. uberis* was found in 16 (51.6%) cases, followed by NAS in 8 (25.8%) cases, and *E. coli* in 5 (16.1%) cases. As a no growth, 2 (6.5%) cases were recorded. *Staphylococcus aureus* and *S. agalactiae* were not recorded during the study. In 22 (71.0%) cases of non-severe CM with *S. uberis* and NAS antimicrobial treatment was used, whereas cases with *E. coli* infection and no growth cases were untreated according to the protocol. The achieved clinical cure was 30/31 (96.8%) of CM cases, while bacteriological cure was 24/29 (82.8%) of culture positive CM cases. Five (17.2%) CM cases were bacteriologically uncured after antimicrobial treatment. These cases were cured successfully by SDCT. Comparing the consumption of antimicrobials

in the 13 months before and during the study, the average consumption of antimicrobials per CM case was reduced from 3.56 to 2.33 doses.

In herd B, a total of 23 cases of CM were recorded, of these, 20 (87.0%) were mild and 3 (13.0%) moderate cases. Of the 23 samples examined, the causative agent was confirmed by culture in 18 (78.3%) cases. *Streptococcus uberis* was identified in 8 (34.8%) cases, *E. coli* in 8 (34.8%) cases, and NAS in 2 (8.7%) samples. No growth result was recorded in 5 (21.7%) cases. No *S. aureus* and *S. agalactiae* were isolated. Antimicrobial treatment was administered to 7 (30.4%) cases of non-severe CM according to the established protocol for *S. uberis* isolation, while one *S. uberis* and remaining CM cases with NAS, *E. coli* and no growth detection (in total 69.6%) were not treated using antimicrobials.

Clinical cure was achieved in 23/23 (100%), and bacteriological cure in 17/18 (94.4%) quarters. One case of persistent *E. coli* infection was subsequently cured successfully during the dry period. Comparison of antimicrobial consumption in the course of the 13 months of study and during the same period before the study resulted in reduction from 4.45 to 1.83 doses per CM case. Treatment of *S. uberis* mastitis and NAS culture using antimicrobials was effective. However, cases of *E. coli* CM were cured successfully without using any antimicrobials.

Subclinical mastitis

In herd A, a total of 19 quarter samples from 12 cows were collected during the trial. On-farm culture proved the infection in 12 (63.2%) cases, of these NAS were isolated in 7 (36.8%), *S. uberis* in 4 (21.1%), and *E. coli* in 1 (5.3%) sample. Remaining 7 (36.8%) samples were with no growth. In accordance with the protocol, no antimicrobials were used for treatment. Spontaneous cure was approved in 16/19 (84.2%) of affected quarters. Out of 19 cases 3 (15.8%) developed chronic infections with permanently elevated SCC in milk.

In herd B, a total of 35 quarter samples from 16 cows with subclinical mastitis were collected. The number of bacteriological positive was 8 (22.9%) samples. NAS were isolated in 4 (11.4%) cases, *S. uberis* in 2 (5.7%) cases and *E. coli* in 2 (5.7%) samples. The remaining 27 (77.1%) samples were recorded without growth. In 2 cases of *S. uberis* infection antimicrobial treatment was administered with bacteriological cure 2/2 (100%). Spontaneous bacteriological cure in other agents was 5/6 (83.3%). One case of recurrent NAS was cured during dry period. Spontaneous cure of no growth cases was achieved in 13/27 (48.1%) quarters. In total, cure rate of all subclinical mastitis was observed in 20/35 (57.5%) quarters. During the following dry period 10 other cases were cured. Remaining 5 cases of subclinical mastitis without evidence of the causative agent developed in chronic form with elevated SCC in milk.

SDCT

In herd A, a total of 53 cows (212 quarters) were dried-off during the study. Based on data analysis 26 (49.1%) cows were evaluated as the low risk category and were therefore dried-off using only internal teat sealant without antimicrobials. Out of remaining 27 (50.9%) cows 42 quarter milk samples were cultured. Only *S. uberis* (11 quarters), NAS (14 quarters) and mixed *S. uberis* and NAS (3 quarters) were isolated. No growth was recorded in 14 samples collected from 12 cows. Based on culture guided SDCT protocol antimicrobials were used in 28 quarters (13.2%) of 13 cows (24.5%). These results show a significant reduction in antimicrobial usage on quarter level by 86.8% compared to BDCT before the study.

In herd B, 72 cows (288 quarters) were dried-off during the course of study. The low risk category contained 57 (79.2%) cows classified according to data analysis. From 15 (20.8%) cows in high risk category 34 quarter samples were cultured. No growth was

recorded in 21 samples, NAS in 6 samples, *S. uberis* in 4 cases, *E. coli* was identified in 2 cases, and 1 case of *Corynebacterium* spp. was recorded. Out of them 5 quarters (1.7%) of the 2 cows (2.8%) with evidence of *S. uberis* (in 4 quarters) and *Corynebacterium* spp. (1 quarter) infection were selected for SDCT. In comparison to the pre-study period, the number of cows and quarters treated by antimicrobials was decreased by 97.2% and 98.3% respectively.

In both herds, *S. uberis* isolates obtained during the study did not show acquired resistance against benzylpenicilin and amoxicillin. Resistance was detected in some NAS species (*S. haemolyticus* and *S. capitis)* in herd A but not in the most common isolates (*S. chromogenes* and *S. simulans)*. No acquired resistance in NAS isolates was recorded in herd B.

Discussion

On farm culture system undoubtedly improves the level of routine diagnosis of IMI (Royster et al. 2014; Ganda et al. 2016; Ferreira et al. 2018; Sipka et al. 2021). Primary goal is the demonstration of a single causative agent, which facilitates on-farm interpretation of results and allows to use modern principles for antimicrobial treatment but also alternative strategies in cases of clinical mastitis when the use of antimicrobials is not necessary (Lago et al. 2011a; Fuenzalida and Ruegg 2019a, b).Therefore, an important part of the mastitis management used in our study were the treatment protocols establishing antimicrobial choices based on culture-proven causative agents and appropriate clinical course of mastitis (non-severe cases). We have also considered the results of antimicrobial MIC determination obtained previously and the cow's health history. Protocols proposed and used did not include the 3rd and 4th generation cephalosporins or any of the fluoroquinolones registered for mastitis treatment.

In both herds, we isolated only environmental, not contagious agents from CM cases. This facilitated the management of targeted therapy in practice as well as chance to achieve higher efficacy. For the treatment of CM caused by *S. uberis*, we used the first-line treatment including amoxicillin as recommended (Regulation EU 2019). Spontaneous cure of CM caused by *S. uberis* cannot be expected to occur at a high rate. Therefore, the treatment of *S. uberis* infections requires the use of the bactericidal effect of penicillins on rapidly dividing cells of the causative agent in the acute phase of inflammation (Deluyker et al. 1999; Hillerton and Kliem 2002; Hoe and Ruegg 2005). The drug dose in the intramammary preparation, which was administered within 3 days, resulted in effective clearance of the causative agent. The level of bacteriological clearance achieved was consistent with the results of other studies and testified to the correctness of the treatment procedure (Lago et al. 2011a, Lago et al. 2016; Bazzanella et al. 2020). In contrast, there are somewhat inconsistent views on the use of antimicrobial treatment for NAS-induced mastitis, due to an expectation of a spontaneous cure rate for 55–60% (Pinzón-Sánchez et al. 2011). In our study, we used antimicrobials to treat some NAS caused CM cases to reach better bacteriological cure rate in accordance with Svennesen et al. (2023). From the point of view of the proven incidence of amoxicillin resistance due to production of beta-lactamases in some NAS isolates, the primary choice was to use the combination of amoxicillin with clavulanic acid.

In contrast, we have never used antimicrobials for the treatment of non-severe CM infections caused by *E. coli* because of the evidence of a high percentage of spontaneous cure (Suojala et al. 2013; Fuenzalida and Ruegg 2019b). Such measure enables to reduce the consumption of antimicrobials and also the emergence and spread of acquired resistance of *E. coli* against several groups of antimicrobials in the herd. Severe CM (grade 3) were not included, similar to other studies (Lago et al. 2011a) due to the necessity

of immediate initiation of systemic antimicrobial treatment without knowledge of the causative agent (Oliveira ang Ruegg 2014) to mitigate the risk of bacteraemia and subsequently septicaemia (Brennecke et al. 2021). However, culture diagnostic test should be done in all severe cases. The test result consequently enables revision of the initial treatment decision (de Jong et al. 2023). A highly debated issue is CM without bacteriological findings, which may be up to 30% samples depending on the culture system used (Lago et al. 2016). In our study similarly with McDougall et al. (2021), the number of no growth results was low, which can be explained by the adherence to the timeliness of sampling just on the day when a new case of CM was detected. The explanation for higher frequency cases of negative culture results is mainly due to actually low number of viable cells of the pathogen in the sample, which is below the detection limit of the system used (Dohoo et al. 2011), or also by late sampling when the spontaneous clearance of the agent occurs due to immunological response in mammary gland.

The success of treatment of clinical mastitis is usually judged in practise by clinical clearance or, more exactly by bacteriological cure, approved by no growth in control sample. The fact that treatment of infections caused by *S. uberis* strains was bacteriologically ineffective in several cases, is not surprising. The success of treatment is not only determined by the appropriate choice of antimicrobials and the susceptibility of the causative organism, but also by the dose and duration of the treatment. It is true that by using the concept of treatment based on cultivation, treatment is initiated on the second day and not immediately after the detection of CM. However, treatment initiated without knowledge of the causative agent on the first day could mean a change in the choice of antimicrobial according to the culture results, or to stop treatment after 24 h in the case of detection of the gram-negative pathogens (*E. coli*) or culture negative results. In these circumstances, it represents a delay of approximately 20-24 h in the start of rational antimicrobial therapy, leading to a reduction in antimicrobial consumption and the risk of induction and spreading of resistance in *E. coli* and other gram-negative bacteria.

Management of subclinical mastitis based on objective data analysis, culture diagnostics of non-chronic cases and the treatment of major mastitis pathogens including environmental streptococci, may lead to a higher bacteriological cure rate (De Vliegher et al. 2018). In our study we did not diagnose any case of *S. aureus* or *S. agalactiae* infection, therefore the treatment decision was made in chosen *S. uberis* IMI in which high bacteriological cure was confirmed in accordance with McDougall et al. (2022).

Furthermore, successful adoption of SDCT into the herd management can contribute to the reduction of antimicrobial usage and risk of development of acquired resistance without the negative risk on herd health outcomes (Bauman et al. 2018; Wittek et al. 2018; Lipkens et al. 2023) as we have confirmed in our study, reaching the high cut in antimicrobial consumption on quarter level.

Antimicrobial consumption in conditions of complex mastitis management using OFC and data analyses was evaluated. On-farm culture system provides the necessary data for target treatment of non-severe CM, subclinical mastitis and SDCT. The high potential for the reduction of the antimicrobial consumption during long term period was proved. OFC system enables continuous monitoring of mastitis pathogens and can be used for evaluation of the effectiveness of antimicrobial treatment and bacteriological cure of clinical and subclinical and SDCT.

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