Comparison of an evidence-based and a conventional mastitis therapy concept with regard to cure rates and antibiotic usage

E.M. Mansion-de Vries[1,2], J. Lücking[1,2], N. Wente[1], C. Zinke[1], M.Hoedemaker[2], V. Krömker[1]

[1] University of Applied Sciences and Arts, Faculty II, Microbiology, Heisterbergallee 12, D-30453 Hannover, Germany
[2] Clinic for Cattle, Hanover University of Veterinary Medicine, Foundation, Bischofsholer Damm 15, D-30173 Hannover, Germany

Date submitted:24/02/2016 *Date accepted: 06/06/2016* *Volume/Page(s): 69/27-32*

Abstract

In milk production, mastitis therapy accounts for the largest proportion of antibiotic use. Numerous studies have suggested that with a differentiated therapy based on mastitis causing pathogens and animal individual variables (regarding the number of lactation, somatic cell count (SCC) and the number of pre-treatments) the amount of antibiotics could be significantly lowered.

The aim of this study was to investigate whether the establishment of an evidence-based mastitis therapy (EBMT) concept could reduce the amount of applied antibiotics compared to a conventional therapeutic (CT) approach with similar curing success. In the EBMT concept the therapy is tailored - with the additional help of on farm culture in the form of Petrifilms™ - to the pathogen and to the patient and includes the latest scientific knowledge. In the CT approach the therapy is only tailored to the patient. The decision concerning therapy depends basically on the knowledge of the therapist and the severity of mastitis symptoms.

To this end, from February until December 2012 all of the approximately 950 cows on a conventional dairy farm in Saxony-Anhalt, Germany, with clinical mastitis cases were assigned to an EBMT- (n = 236 cows) and a CT-group (n = 230 cows) based on the ear tag number and treated accordingly. Subsequently, the results of the two treatment groups were evaluated with respect to the clinical cure (CC), the bacteriological cure (BC), the full cure (FC), the relapse and culling rate and the amount of local and parenteral antibiotics used. Furthermore, the mean costs per clinical mastitis case of these two therapeutic concepts were compared.

There was a significantly higher CC in the EBMT- versus the CT-group with simultaneous significant reduction in the local antibiotic doses without negative influence on the BC, FC, relapse and culling in the EBMT-group. Also, the mean costs per clinical mastitis case were significantly lower in the EBMT-group.

This pilot study showed that by implementing on farm culture the use of an EBMT concept significantly reduces the use of local antibiotics in mastitis therapy without having any negative significant changes in the therapy outcome or economic aspects.

Introduction

Mastitis is the most common and most costly dairy cattle disease; its therapy also causes the greatest antibiotic use in milk production. Due to the time-consuming standard diagnostics the treatment decision is usually made without knowledge of the pathogen. Furthermore, it is merely based on the practical experience of the veterinarian or the herd manager taking clinical symptoms into account [1]. Studies have shown that treatment of mastitis should be selected according to the pathogen [2, 3, 4, 5]. Mastitis, caused by coliforms, requires, in certain circumstances, no antibiotic therapy [2, 3, 6, 7, 8], whereas the presence of a Gram-positive pathogen usually requires the use of local antibiotics [2, 9]. Only for febrile mastitis caused by coliforms does a parenteral antibiotic seem to be necessary because of its high likelihood of becoming septical [10].

The therapy outcome is influenced not only by the pathogen but also by patient-related factors. The probability of cure depends on factors such as the age and lactation number of the cow [3, 11], DIM and the number of previous mastitis cases [12]. Taking into account the characteristics of the mastitis-causing pathogen, the patient and the continuous incorporation of new scientific knowledge in the treatment decision, mastitis therapy would be more evidence-based [13]. Implementation of an evidence-based therapy concept could result in a reduction in antibiotic use in mastitis therapy without having any detrimental influence on the treatment results.

The aim of this present study is to provide an evidence-based mastitis therapy (EBMT) approach additionally using an on farm culture and to discuss the results of its implementation as compared to one conventional therapeutic (CT) approach.

Materials and Methods

Farm, animals and mastitis definition:

The randomised, prospective, clinical study was conducted from February to December 2012 on a dairy farm in Saxony-Anhalt, Germany. It is a conventional, commercially oriented farm with cows housed in free-stalls. Herd size was approximately 950 lactating cows of the Holstein Friesian breed. Annual milk production averaged 9,300 kg (FECM) and bulk tank milk somatic cell counts (SCC) ranged from 200,000 to

Table1: Assignment of patients involving the mastitis severity, the result of the Petrifilm™ concept and the therapy worthiness for one of 6 experimental antibiotic therapies in the evidence-based mastitis therapy (EBMT)-group

Therapy group	Antibiotic treatment	Selection criteria			References
		Result of the Petrifilm™ concept (PC)	Mastitis severity (MS)	Therapy worthiness (TW)	
1	No antibiotics	GN[1] and NMG[2]	1 and 2	TU[4], Y[5] and R[6]	Guterbock et al., 1993; Roberson et al., 2004; Suojala et al., 2010
		GP[3]	1 and 2	TU	Owens et al., 1999; Østerås, 2006
2	Antibiotics i.m.	GN and NMG	3	TU, Y and R	Wenz et al., 2001; Erskine et al., 2002
		GP	3	TU	Erskine et al., 2002
3	1.5 days antibiotics i.mam.	GP	1 and 2	R	Hillerton & Kliem, 2002; Roberson et al., 2004
4	5 days antibiotics i.mam.	GP	1 and 2	Y	Sol et al., 2000; Krömker et al., 2010
5	Antibiotics i.m.and 1.5 days i.mam.	GP	3	R	Hillerton & Kliem, 2002; Roberson et al., 2004; Erskine et al., 2002
6	Antibiotics i.m.and 5 days i.mam.	GP	3	Y	Sol et al., 2000; Krömker et al., 2010; Erskine et al., 2002

[1] Gram-negative
[2] no significant microorganism growth
[3] Gram-positive
[4] Therapy unworthy cows = cows with three or more pre-treatments in the affected quarter in the current lactation, palpable changes in udder parenchyma or more than 700,000 cells / ml milk in the individual cow SCC of the previous three months
[5] Young therapy worthy cows = cows in the first or second lactation where the definition of the TU does not apply
[6] Remaining therapy worthy cows = cows where the definitions of TU and Y do not apply

250,000 cells / mL milk. The clinical mastitis incidence was about 5% of the milking cows per month. The animals were milked three times a day.

The criteria for farm selection were the willingness of the owner to cooperate, the monthly participation in dairy herd improvement (DHI) to record individual cows' SCC and an udder health management which is satisfactory and consistent in practice. Forestripping of each cow was done by trained personnel to detect abnormalities in milk. An animal indicated signs of clinical mastitis if one or more quarters showed changes in the appearance of milk (colour, viscosity, consistency) and/or local clinical signs (swelling, heat, pain of the udder) with or without associated general clinical signs. The affected animals were separated after milking and examined with regard to fever and general condition. After clinical examination of the animals a classification of mastitis into mastitis severity score (MS) 1 (only change in the appearance of milk), MS 2 (MS 1 and clinical sign on the udder) or MS 3 (MS 2 and fever) [14] followed. At this time cows with clinical mastitis were randomly assigned to the EBMT- and the CT-group according to the ear tag number; animals with an even ear tag number were assigned to the CT-group, animals with an odd ear tag number to the EBMT-group. During the experimental period, all animals with clinical mastitis participated in the study provided they were not suffering from another disease at this time, or had a teat injury.

Sampling and Petrifilm™ concept as an on farm culture test:

Immediately after detecting clinical mastitis a duplicate foremilk sample of the affected quarter was taken antiseptically [15]. Post-treatment quarter milk samples were taken antiseptically at 14 (± 1) and 21 (± 1) days post diagnosis. All samples were collected as described by NMC [16] and stored and handled at a maximum of 8 °C. Twice a week one of the duplicate mastitis milk samples and all post treatment samples were transported to the microbiological laboratory of the University of Applied Sciences and Arts Hannover, Germany, where the samples were examined by conventional microbiological diagnostics according to the GVA [15]. From cows of the EBMT-group the second of the duplicate samples was analysed on the farm immediately after collection using AerobicCount-and ColiformCount-Petrifilm™ plates (3M, Neuss, Germany) [17]. The Petrifilm™ plates were incubated at 37 °C. After 24 hours, the plates were examined for bacterial growth. Enumeration and interpretation of the Petrifilm™ plates allow a classification of milk samples with respect to Gram-positive (GP), Gram-negative (GN) and no significant microorganism growth (NMG) [18].

Evidence-based mastitis therapy concept (EBMT-concept):

The therapeutic concept of the EBMT-group is based on numerous research results of a literature review, which were combined in an overall concept (Table 1). The therapeutic decision is based on first the MS, second the results of an on farm culture using the Petrifilm™ concept (PC) and third the therapy worthiness (TW) of affected animals. Referencing the number of pre-treatments in the affected quarter, the individual cows' SCC in the three preceding DHIs and the number of lactation, the animals were assigned to one of three groups with respect to the TW:

1. Therapy unworthy cows (TU) are cows with already three or more treatments in the affected quarter in the current lactation, palpable changes in udder parenchyma or more than 700,000 cells / mL milk in the previous three DHIs [19, 20]
2. Young therapy worthy cows (Y) are cows in the first or second lactation where the definition of TU does not apply.
3. Remaining therapy worthy cows (R) are cows where the definitions TU and Y do not apply.

Treatment of the EBMT-concept:

All animals in the EBMT-group received an NSAID. A decision concerning the antibiotic treatment depended on the MS, the PC and the TW (Table 1). According to the MS, PC and TW the animals were assigned to one of 6 experimental therapies:

1. Only NSAID
2. NSAID + parenteral antibiotherapy,

Table 2: Written treatment instructions of the vet for the conventional therapeutic (CT)-group

Treatment	Mastitis severity 1	Mastitis severity 2	Mastitis severity 3
Local antibiotherapy	75 mg ampicillin + 200mg cloxacillin (Gelstamp®75mg/200mg, Zoetis, Berlin, Germany) 3-10 times every 12 hours	75 mg cefquinome (Cobactan® LC, MSD, Animal Health, Unterschleißheim, Germany) 3 times every 12 hours OR: 75 mg ampicillin + 200mg cloxacillin (Gelstamp® 75mg/200mg, Zoetis, Berlin, Germany) 3-10 times every 12 hours	75 mg cefquinome (Cobactan® LC, MSD, Animal Health, Unterschleißheim, Germany) 3 times every 12 hours
Parenteral antibiotherapy	No parenteral antibiotherapy	No parenteral antibiotherapy	1 mg/kg body weight cefquinome (Cobactan® 2.5% , MSD, Animal Health, Unterschleißheim, Germany) 3 times once daily
NSAID	No NSAID	No NSAID	0.5 mg/kg body weight Meloxicam (Metacam® 20 mg/mL, Boehringer Ingelheim Pharma GmbH & Co. KG, Boehringer Ingelheim, Germany) subcutaneously once OR: 3 mg/kg body weight ketoprofen (Romefen® 10%, Merial, Hallbergmoos, Germany) intramuscularly once daily up to 3 days

3. NSAID + 1.5-day local antibiotherapy
4. NSAID + 5-day local antibiotherapy
5. NSAID + parenteral + 1.5-day local antibiotherapy
6. NSAID + parenteral + 5-day local antibiotherapy

As parenteral antibiotherapy 1 mg/kg body weight cefquinome (Cobactan® 2.5 %, MSD, Animal Health, Unterschleißheim, Germany) was administered intramuscularly for three days once daily. As a local antibiotherapy 75 mg cefquinome (Cobactan® LC, MSD, Animal Health, Unterschleißheim, Germany) was administered intramammarily every 12 hours. 3 mg/kg body weight ketoprofen (Romefen® 10 %, Merial, Hallbergmoos, Germany) was administered intramuscularly as NSAID for up to three days once daily. In each case, initial therapy included the administering of an NSAID. In the case of MS 3 a parenteral antibiotherapy with cefquinome (Cobactan® 2.5 %, MSD, Animal Health, Unterschleißheim, Germany) was additionally administered intramuscularly. A decision concerning local antibiotic treatment was made after receiving the PC-outcome, i.e. 1 day after diagnosis.

Conventional therapeutic concept (CT-concept):

The animals in the CT-group were treated on the basis of written treatment instructions from the vet which were took into consideration scoring of clinical symptoms as well as his practical experience, including the drugs withdrawal period (Table 2). This was at the beginning of the study the standard therapy used on the farm.

In general, the duration of therapy amounted to three days. In cases of persistent clinical symptoms therapy was prolonged. Animals with MS1 [14] mostly received only local therapy with penicillin (Gelstamp®, Zoetis, Berlin, Germany) every 12 hours with the lowest possible withdrawal period (Table 2). Upon the occurrence of MS 2[14], the animal was generally treated locally with 75 mg cefquinome (Cobactan® LC, MSD, Animal Health, Unterschleißheim, Germany) or with penicillin (Gelstamp®, Zoetis, Berlin, Germany) every 12 hours (Table 2). In the presence of febrile mastitis the animals received a parenteral treatment with 1 mg/kg body cefquinome (Cobactan® 2.5 %, MSD, Animal Health, Unterschleißheim, Germany) intramuscularly up to three days once daily in addition to local cefquinome therapy. Depending on the assessment of the herdmanager an NSAID (Metacam® 20 mg/mL, Boehringer Ingelheim Pharma GmbH & Co. KG, Boehringer Ingelheim, Germany or Romefen® 10 %, Merial, Hallbergmoos, Germany) or homeopathic fever reducer (Pyrogenium compositum inject, Dr. Schaette, Bad Waldsee, Germany) was administered sporadically (Table 2).

Definitions of the outcome variables, cost calculations and documentation:

Clinical cure (CC) was defined as the absence of any clinical signs of mastitis on the affected quarter 5 days after diagnosing clinical mastitis. Bacteriological cure (BC) was certified when the mastitis-causing pathogen was absent in both post-treatment samples 14 and 21 days after diagnosis. A case was considered to be fully cured (FC) if in addition to CC and BC the cell count was below 200,000 cells/mL milk in both control samples. A relapse was defined as detection of a renewed clinical mastitis in the treated quarter within 100 days after diagnosis of the original case. It was documented when the animal was culled within 100 days after diagnosis due to mastitis in the treated quarter.

In the calculation of the mean costs per clinical mastitis case the following aspects were included:

Costs for drugs, costs for the on farm culture and costs for milk money losses as a result of having to wait for ordered drugs. The costs for the on farm culture included costs for the Petrifilms™, costs for extra time due to performing the Petrifilm™ concept and costs for the equipment. Only in the EMBT-group were costs for the on farm culture incurred due to the additional use of the Petrifilm™ concept.

At clinical mastitis case level, all data were either recorded cow-side onto data capture forms or retrieved onto data forms from on farm software at the time of treatment. The amount of local and parenteral antibiotics used per clinical mastitis case was, however, recorded onto data capture forms. All data per case were rounded up in an Excel table (Microsoft Corporation, Redmond, Washington, USA).

Statistical analysis:

To test for homogeneity of data of the EBMT- and CT-group, normally distributed metric data were tested statistically with a Student's t-test. Nominal data, i.e. clinical cure, were compared as proportions with a χ^2-test.

The affected quarter was the unit of observation. BC, CC and FC, relapse and culling were evaluated using mixed model logistic regression analysis where parity, pathogen group (no growth, *Escherichia (E.)coli* and coliforms, *Streptococcus uberis*, contaminated [more than two pathogens in one sample], staphylococci, mixed infections and other), days in milk (DIM) as a continuous covariate, MS category (1, 2 or 3) and treatment category (EBMT or CT-group) were included as fixed

effects and the cow was included as random effect. Goodness of fit of models was assessed by using the Akaike's Information Criterion. After identifying a positive definite Hesse matrix model assumptions of the final models were checked by plotting deviance residuals against fitted values.

For the statistical analysis, SPSS (SPSS 23.0, IBM Corp., Armonk, USA) was used. The full model, including interaction terms, was given by:

Logit (BC, CC, FC, relapse, culling) = Lactation + DIM + pathogen-group + MS + treatment category + MS x treatment + cow (random) + e

A value of $P < 0.05$ was considered significant.

Results

A total of 466 clinical mastitis cases were enrolled in the study (230 in the CT- and 236 in the EBMT-group [Table 3]). 77 mastitis cases were excluded beforehand due to missing Petrifilm™ results, incorrect assignment to the treatment groups or lack of control samples. The test groups were similar in terms of DIM (P=0.49), lactation (P=0.82) and mastitis-causing pathogen distribution (P=0.92) (Table 3). *E. coli* was the most frequently detected pathogen (Table 4). Only the distribution of MS showed significant differences (P=0.012) between the experimental groups (Table 3). The resulting possible effects on the outcome variables were examined.

Table 3: Descriptive data of composition of the evidence-based mastitis therapy (EBMT)-group and conventional therapeutic (CT)-group

	EBMT-group	CT-group	P-value
Mastitis cases [n]	236	230	
DIM [median]	136 (± 101)	129 (± 99)	0.49
Lactation number [median]	3 (+3/-2)	3 (+10/-2)	0.82
Mastitis severity 1 [n]	50	77	
Mastitis severity 2 [n]	138	114	0.012
Mastitis severity 3 [n]	48	39	

No differences in BC, FC, relapse, or culling rate existed between the EBMT- and the CT-group (Table 5). The CC rate in the EBMT-group was significantly higher (Table 5, OR=1.67; CI: 1.11-2.52) than in the CT-group.

Table 4: Microbiological results of the evidence-based mastitis therapy (EBMT)-group and conventional therapeutic (CT)-groupbased on conventional microbiological diagnostics method (n = 466, clinically mastitic milk samples)

Microbiological findings	n	%
Coliforms other than *E. coli*	6	1.3
E. coli	102	21.9
CNS[1]	10	2.1
Contaminated[2]	41	8.8
No growth	146	31.3
S. aureus	17	3.6
Sc. uberis	73	15.7
Other	61	13.1
Mixed growth[3]	10	2.1
Total	**466**	**100.0**

[1] Coagulase-negative staphylococci
[2] More then two different pathogens were detected in one sample
[3] Two different pathogens were detected in one sample

The evaluation of the administered antibiotic doses showed a significantly lower dose of local antibiotics in the EBMT-group (Table 5). On average, 5.39 (CI: 4.56 - 6.22) more doses of local antibiotics were administered per case in the CT-group. Regarding the use of parenteral antibiotics no differences were observed (P=0.2).

The calculation of the costs per clinical mastitis case showed significantly lower therapy costs in the EBMT-group (Table 5). The costs for the Petrifilm™ concept amounted to €5.50.

Discussion

Due to the period which elapsed until the bacteriological test results were available, decisions concerning clinical mastitis treatment were made without any knowledge of the respective mastitis-causing pathogen. This study describes a comparison of a CT (without using bacteriological findings) with an EBMT concept where the result of an on farm culture is one part of the differentiated therapeutic decision. Numerous studies describe the existence of some on farm test methods which provide results within 24 hours [17, 18]. Their application would provide quicker information about the mastitis-causing

Table 5: Descriptive data of treatment effects, mean antibiotic doses and costs per mastitis case of the evidence-based mastitis therapy (EBMT)-group and conventional therapeutic (CT)-group (most parameters were compared with a χ^2-test or Student's t-test)

	EBMT-group		CT-group		P-value
	n	%	n	%	
Clinical cure rate	148/236	62.7	124/230	53.9	0.014
Bacteriological cure rate	98/144	68.1	79/121	65.3	>0.05
Full cure rate	13/142	9.2	12/121	9.9	>0.05
Relapse	83/236	35.2	80/230	34.8	>0.05
Culling rate	22/236	9.3	24/230	10.4	>0.05
Mean doses of local antibiotics (sd[1])	3.08 (± 4.53)		8.47 (± 4.60)		<0.0001
Mean doses of parenteral antibiotics (sd)	0.69 (± 1.49)		0.53 (± 1.20)		>0.05
Mean costs per mastitis case in € (sd)	96.60 (± 70.29)		138.20 (± 68.86)		< 0.0001

[1] Standard deviation

pathogens, thus enabling a target-oriented therapy. The present study demonstrated that using an EBMT-concept significantly reduces local doses of antibiotics and economic costs per mastitis case. Adverse effects on the cure, relapse and culling rates were not found. Additionally using an on farm culture enables not only adapting the therapy to the patient (number of lactation and MS) but also to the mastitis-causing pathogens. When no significant microorganism growth or Gram-negative bacteria was detected, therapy was performed without antibiotics. Despite not performing antibiotic therapy no significant lower BC or FC rates were observed as described in previous studies [2, 8].

In terms of CC rate significantly higher healing rates were observed in the EBMT-group. This is presumably due to the administering of ketoprofen to all patients in the EBMT-group. In contrast, only in 30% of the mastitis cases in the CT-group was an NSAID administered. Other authors like Shpigel *et al.* [21] were also able to demonstrate the positive effect of NSAIDs on the CC.

The evaluation of the economic aspects per clinical mastitis case showed significantly lower costs in the EBMT-group of about €40. Despite additional costs in the EBMT-group for the Petrifilm™ concept of €5.50 and a general administering of an NSAID this could be achieved. The additional costs in the EBMT-group could be compensated by lower costs due to shorter withdrawal periods for ordered drugs and savings on local antibiotic doses.

It should be mentioned that the EBMT concept requires slightly more time than the CT concept. In the EBMT concept more time is needed for taking milk samples of every clinical mastitis case, for doing the Petrifilm™ concept and for observing the clinical mastitis case more intensively as some cows were treated without antibiotics. This implicates the risk of a worsening degree of mastitis severity and of complications. The additional time required to perform the Petrifilm™ concept was allowed for in the calculation but the other additional time was not.

As the decision on the therapy significantly depends on the outcome of the Petrifilm™ concept, it was not possible, nor desired to blind the study. Therefore, the veterinarian and the herdsman knew the results of both, PC and therapy group. This could have had an influence on the assessment of CC.

Since this study was conducted only on one farm, over a quite short period of 11 months, it must be regarded as a pilot study. The results cannot be transferred to other herds in general but the concept can. To determine whether the results are repeatable in other herds with different pathogen distributions, further investigations have to be carried out.

Conclusions

The pilot study presents an evidence-based mastitis therapy concept in comparison to a conventional mastitis therapy concept. The described results show that the implementation of the evidence-based mastitis therapy concept significantly reduces the use of local antibiotics in mastitis therapy without having any significant changes in the therapy outcome. Despite extra time for doing the evidence-based concept its outcome seemed to be interesting in terms of economic aspects.

Conflicts of interest

None declared.

Acknowledgements

The authors wish to thank Dr. Matzke and Ohreland KG for providing their cows and enabling the study to be carried out. Furthermore, we are grateful to 3M for supplying the Petrifilm™ plates.

References

1. Neeser NL, Hueston WD, Godden SM, Bey RF. Evaluation of the use of an on-farm system for bacteriologic culture of milk from cows with low-grade mastitis. J Am Vet Med Assoc. 2006; 228: 254-60.
2. Roberson JR, LD Warnick, G Moore. Mild to moderate clinical mastitis: Efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent milk-out treatment versus no treatment. J Dairy Sci. 2004; 87: 583-592.
3. Pyörälä SH, EO Pyörälä. Efficacy of parenteral administration of three antimicrobial agents in treatment of clinical mastitis in lactating cows: 487 cases. J Am Vet Med Ass. 1998; 212: 407-412.
4. Erskine RJ, PC Bartlett, JL Van Lente, CR Phipps. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. J Dairy Sci. 2002; 85: 2571-2575.
5. Krömker V, JH Paduch, D Klocke, J Friedrich, C Zinke. Wirksamkeit einer verlängerten Arzneimittelapplikation zur Behandlung mittel- hochgradiger klinischer Mastitiden bei Milchkühen. Ber Münch Tierärztl Wochenschr. 2010; 123: 147-152.
6. Guterbock WM, AL Van Eeenennaam, RJ Anderson, IA Gardner, JS Cullor, CA Holmberg. Efficacy of intramammary antibiotic therapy for treatment of clinical mastitis caused by environmental pathogens. J Dairy Sci. 1993; 76: 3437-3444.
7. Pyörälä S, L Kaartinen, H Käck. Efficacy of two therapy regimens for treatment of experimentally induced *Escherichia coli* mastitis in cows. J Dairy Sci. 1994; 77: 453-461.
8. 8. Suojala L, H Simojoki, K Mustonen, L Kaartinen, S Pyörälä. Efficacy of enrofloxacin in the treatment of naturally occurring acute clinical *Escherichia coli* mastitis. J Dairy Sci. 2010; 93: 1960-1969.
9. Hillerton JE, KE Kliem. Effective treatment of *Streptococcus uberis* clinical mastitis to minimize the use of antibiotics. J Dairy Sci. 2002; 85: 1009-1016.
10. Wenz JR, GM Barrington, FB Garry, KD McSweeney, RP Dinsmore, G Goodell, RJ Callan. Bacteremia associated with naturally occurring acute coliform mastitis in dairy cows. J Am Vet Med Assoc. 2001; 219: 976-981.
11. Sol J, OC Sampimon, HW Barkema, YH Schukken. Factors associated with cure after therapy of clinical mastitis caused by *Staphylococcus aureus*. J Dairy Sci. 2000; 83: 278-284.
12. Pinzón-Sánchez C, PL Ruegg. Risk factors associated with short-term post-treatment outcomes of clinical mastitis. J Dairy Sci. 2011; 94: 3397-3410.
13. Sackett DL, WM Rosenberg, JA Muir-Gray, RB Haynes. Evidence based medicine: what it is and what it isn't. Brit Med J. 1996; 312: 71-72.
14. International Dairy Federation (IDF). Suggested interpretation of mastitis terminology. Bulletin of the IDF. 1999; 338, Brussels.
15. German Veterinary Association (GVA). Guidelines for antiseptic milk sampling and guidelines to isolate and identify mastitis pathogens. GVA. 2009; Gießen, Germany. [Deutsche Veterinärmedizinische Gesellschaft (DVG). Leitlinien zur Entnahme von Milchproben unter antiseptischen Bedingungen und Leitlinien zur Isolierung und Identifizierung von Mastitiserregern. GVA. 2009; Gießen, Deutschland.]
16. National Mastitis Council (NMC). Laboratory Handbook on Bovine Mastitis. NMC. 1999; Madison, WI.

17. Mansion-de Vries EM, N Knorr, JH Paduch, C Zinke, M Hoedemaker, V Krömker. A field study evaluation of Petrifilm™ plates as a 24-h rapid diagnostic test for clinical mastitis on a dairy farm. Prev Vet Med. 2014; 113(4): 620-624.
18. McCarron J, G Keefe, S McKenna, I Dohoo, D Poole. Laboratory evaluation of 3M Petrifilms and University of Minnesota Bi-Plates as potential on-farm tests for clinical mastitis. J Dairy Sci. 2009; 92: 2297-2305.
19. Owens WE, SC Nickerson, CH Ray. Efficacy of parenterally or intramammarily administered tilmicosin or ceftiofur against *Staphylococcus aureus* mastitis during lactation. J Dairy Sci. 1999; 82: 645-647.
20. Østerås O. Mastitis epidermiology - Practical approaches and applications. 24. World Buiatrics Congress. 2006; Nizza: 203-215.
21. Shpigel NY, M Winkler, A Saran, G Ziv, F Longo. Anti-inflammatory ketoprofen in the treatment of field cases of bovine mastitis. Res Vet Sci. 1994; 56: 62-68.