



Original Article

## Blitz therapy in control of *Streptococcus agalactiae* subclinical mastitis in dairy cows reduces somatic cells count of bulk milk tank in a dairy cattle herd

Letícia Caldas Mendonça<sup>1</sup>, Marianna Barbosa Gentilini<sup>2</sup>, Nara Ladeira Carvalho<sup>3</sup>, Victor Marco Rocha Malacco<sup>4</sup>, Camila Flávia de Assis Lage<sup>4</sup>, Lívio Ribeiro Molina<sup>2</sup>

<sup>1</sup> Embrapa Gado de Leite, Juiz de Fora, MG, Brazil.

<sup>2</sup> Departamento de Clínica e Cirurgia Veterinárias da Escola de Veterinária da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.

<sup>3</sup> Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (USP), Pirassununga, SP, Brazil.

<sup>4</sup> Departamento de Zootecnia da Escola de Veterinária da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil.

### ARTICLE INFO

#### Article history

Received 15 June 2016

Received in revised form 15 December 2016

Accepted 07 February 2017

#### Keywords:

Milk quality

Treatment

Mammary gland

SCC

### ABSTRACT

The objective of this study was to assess the control feasibility of *Streptococcus agalactiae* subclinical mastitis and its impact in reducing somatic cell counts (SCC) of bulk milk tank. During the lactation, 33 cows positive for *Streptococcus agalactiae* in microbiological culture were treated with three applications of intramammary antimicrobial containing 100.000 UI of penicillin and 150 mg of novobiocin, in an interval of 12 hours. Individual and bulk tank milk samples were collected for somatic cell count (SCC) analysis and milk yield was measured before and after treatment. Statistical analyses were performed with SAS System, version 9.2. All cows treated against *S. agalactiae* had microbiological cure and reduction of SCC, indicating the effectiveness of treatment. SCC in bulk tank was reduced from 829,000 to 513,000 cells/ml.

### INTRODUCTION

Mastitis caused by *Streptococcus agalactiae* is extremely costly once these bacteria usually infect many quarters of the mammary gland, causing extensive damage to alveolar cells and resulting in decrease of milk production (EDMONDSON, 2011; KEEFE, 2012). The reduction of intramammary infection rates, and consequently the reduction in somatic cell count (SCC), adds great economic value to the milk produced, and allows great profit to the farmers due to the improvement of dairy industries

efficiency, also ensuring quality and safety of dairy products offered to consumers (KLEI; YUN, 1998).

The reduction of total bacterial count (TBC) and SCC has become a common goal since the implementation of programs to improve milk quality and the need of dairy industries of high milk quality (BRASIL, 2012; ALVES et al., 2014). In this context, the subclinical mastitis treatment during the lactation can be considered an alternative in herds with high prevalence of *S. agalactiae* infection (TYLER et al., 1992; REYES et al., 2015). In these herds, selecting cows eligible to the blitz therapy (i.e. the

\* Corresponding author: [lmolina@vet.ufmg.br](mailto:lmolina@vet.ufmg.br)

antibiotic intramammary treatment into all four quarters during lactation to eradicate *S. agalactiae* infections) through microbiological culture proved to be extremely important and economically feasible (EDMONDSON, 2011; REYES et al., 2015). Additionally, individual SCC and microbiological cultures are essential to monitor prevalence of infections and to evaluate treatments efficacy.

This study aimed to evaluate technical feasibility of controlling *S. agalactiae* and its impact on the quality of milk as SCC, using the treatment of infected cows during the lactation, i.e., blitz therapy.

## MATERIALS AND METHODS

The Animal Ethics Committee of the Universidade Federal de Minas Gerais (protocol n. 117/2011) approved the protocols for this study.

The study was conducted on a dairy farm in the state of Minas Gerais, in a herd with 114 crossbred Holstein x Gyr lactating cows with an average production of 18 kg milk/day. The general management of milking included good hygiene practices and proper review of the milking equipment.

Individual milk samples from all lactating cows were collected for microbiological culture in days 21, 14 and 7, and the day of the treatment was considered day zero. Milk samples were obtained immediately before milking and put into a sterile container, after proper antiseptic procedures at the teat end. Collected material was frozen and sent to laboratory and microbiological culture was done according to Brito and Brito (1999).

Volumes of 10 µl of each sample were seeded with a calibrated, disposable handle in each quadrant of a blood agar plate with 5% of sheep's blood without fibrin, following 35°C incubation during 24 hours, according to Harmon et al. (1990). After the incubation period, reading of first plates was carried out, noting the microbial growth, aspect, color and number of occurring colonies. Subsequently, plates were again incubated at 35°C for another 24 hours, performing the second reading after a total incubation period of 48 hours. Milk samples presenting growing microorganisms, working as a representative colony, were selected, seeded in agar BHI (Brain Heart Infusion – brain and heart agar infusion) and incubated at 35°C for 24 hours. After this period, the isolated microorganisms were microscopically examined with smears colored by Grams technique, and evaluated regarding its catalase production and, then, submitted to identification tests.

## Identification of positive coco gram and negative catalase

This group was identified via the CAMP test (Christie, Atkins e Munch-Peterson), sodium hipurato hydrolysis, growth in presence of 6.5 of sodium chloride, growth in environment containing bile, aesculin and aesculin hydrolysis. The classification was carried out as per Brito and Brito (1999).

Among the 40 animals identified as carriers of *S. agalactiae*, 33 received three consecutive intramammary treatments, with an interval of 12 hours, 10 ml of associated 100.000 UI of penicillin and 150 mg of novobiocin, with prior teats antiseptics performed with cotton soaked in alcohol 70%. The milk produced by these animals was discarded according to the withdrawal period.

Drying off was carried out early in the other seven animals identified as being in an advanced stage of lactation. All treated animals were segregated after treatment, including milking period, until the last result of microbiological culture. As the number of animals identified in the microbiological exam with *S. aureus* was too small (n = 2), they were culled.

Microbiological cultures of milk from treated animals were performed at days 14 and 21. Animals were considered cured if no growth of *S. agalactiae* was observed in culture results after treatment. Individual SCC analysis from all lactating cows was performed at days 7, 14, and 21. Bulk tank milk samples were collected for SCC analysis on days 30 and 21, according to Brito (2001).

## Statistical analysis

'Blitz therapy' effect over time on the individual's somatic cell counts was assessed using a mixed model approach, in order to adequately take into account the dependence among observations over time. Counts were first log-transformed in order to stabilize the variance and obtain approximate normality.

The model employed was

$$Y_{jt} = \mu + \tau_{t} + e_{jt},$$

where  $Y_{jt}$  is the  $\ln(\text{SCC})$  for the  $j$ th cow at the  $t$ th time period;  $\mu$  is the overall mean;  $\tau_{t}$  is the effect of the  $t$ th day relative to treatment; and  $e_{jt}$  is the random error associated with the  $j$ th cow at  $t$ th time point measurement, assumed to be normally distributed with zero mean, constant variance and covariance given by a first-order autoregressive (AR(1)) structure. This was selected among other common covariance structures (variance

components, compound symmetry, unstructured, banded main diagonal, first-order autoregressive, heterogeneous AR(1), first-order ante-dependence, heterogeneous CS and spatial power) by both AIC and BIC criteria, either considering or not the cow effect as random, following the general guidelines given by Milliken and Johnson (2009).

Once the best fitted covariance structure was selected, the effect of time over  $\ln(\text{SCC})$  was assessed by means of Type 3 F test and  $\ln(\text{SCC})$  by means of least squares method for 14 and 21 days after treatment application. They were compared with  $\ln(\text{SCC})$  LS' mean 7 days before the application through the Dunnett-Hsu test, a test designed for comparisons with a control (LITTELL et al., 2006).

The analyses were performed with the MIXED procedure of SAS System, version 9.2 (SAS Institute Inc., 2009) and the graphs were created in R software, version 3.0.0 (R Core Team, 2013). Statistical significance was considered when  $p \leq 0.05$ .

## RESULTS

Thirty-three animals infected with *S. agalactiae* received intramammary antimicrobial treatment and their milk was discarded following recommended withdrawal period. Microbiological culture results from 114 animals enrolled in the study are shown in Table 1.

Table 1 - Microbiological culture results from 114 animals enrolled in the study.

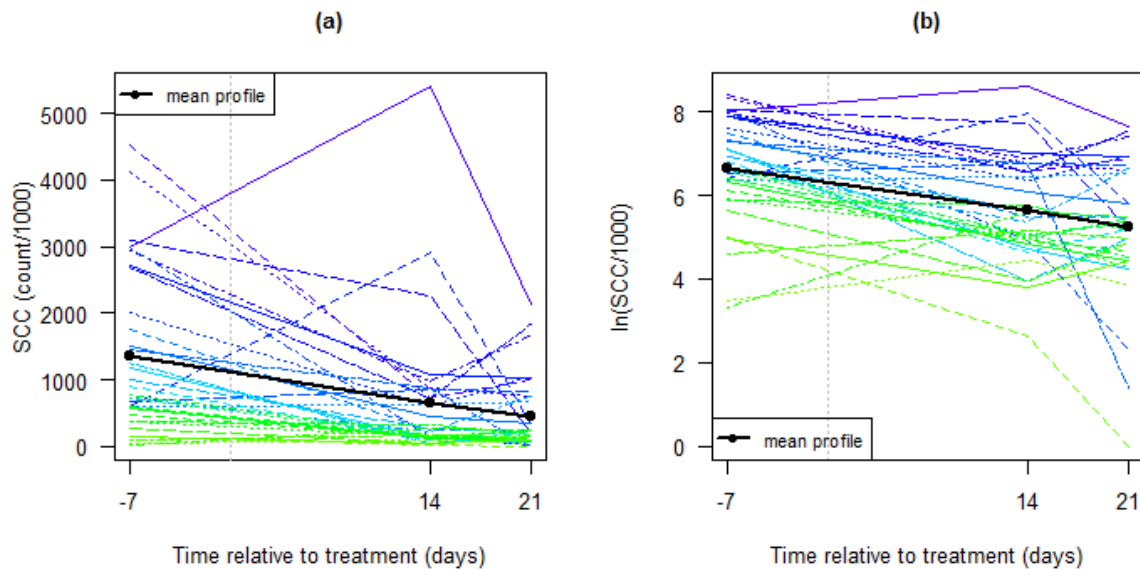
Microbiological culture	Number of animals	Percentage (%)
<i>Streptococcus agalactiae</i>	40	35,1
<i>Streptococcus</i> sp	18	15,8
<i>Coagulase-negative Staphylococci</i>	10	8,8
Multi growth*	8	7,0
Yeast cells	6	5,3
<i>Escherichia coli</i>	4	3,5
<i>Staphylococcus aureus</i>	2	1,8
<i>Bacillus</i> sp	1	0,9
No bacterial growth	25	21,9
TOTAL	114	100

\* More than two species of bacteria

All animals treated with the blitz therapy (n = 33) showed no *S. agalactiae* growth at microbiological culture performed on days 14 and 21 after treatment. 'Blitz therapy' was highly effective in reducing SCC when

comparing average somatic cell count at day 7 before the application with the count means of days 14 and 21 after that application ( $p < 0.0001$  for both comparisons), as it can be clearly seen on Figure 1.

Figure 1 - Somatic cell count (SCC) profiles for the 33 cows considered in the study: (a) counts, (b) log of counts.



Source: author's collection.

SCC in bulk tank assessed the impact of blitz therapy in the months before and after the treatment. SCC in bulk tank, which was 829,000 cells/ml in the month before the blitz therapy, i.e. during a high prevalence of *S. agalactiae* period, was reduced to 513,000 cells/ml after 30 days of treatment. Milk production three months before and three months after the blitz therapy did not change significantly ( $p > 0.05$ ).

## DISCUSSION

All animals treated with blitz therapy ( $n = 33$ ) showed no growth of *S. agalactiae* in microbiological culture performed at days 14 and 21. Control of *S. agalactiae* can be difficult because the infection spreads quickly among cows if the milking hygiene, milking routines or milking machine performance is less than optimal. However, this study presents encouraging results, which can be seen as an alternative tool in order to eradicate *S. agalactiae* in herds with a high prevalence of this pathogen, because the therapy allows animals to be treated during the lactation. As a main advantage, blitz therapy has a fast reduction of subclinical infections and SCC, compared to practices of pre and post dip and dry cow therapy, which results, although excellent, can only be seen in a long term (ERSKINE; EBERHART, 1990). Smith and Ward (1975) reported an infection reduction of 21% to 4.7% in quarters positive for *S. agalactiae*, 20 days after treatment using intramammary penicillin. Goodger et al. (1988) obtained microbiological cure in 97 out of 99 cows treated with cephapirin after three weeks. Efficacy of antibiotics treatment depends on three factors: proper concentration of drug on the site of infection, susceptibility of microorganism to the drug, and host's intact defenses to fight infection (VIANA, 2000). Results show that three consecutive intramammary applications using penicillin and novobiocin were enough to keep antimicrobial minimum inhibitory concentration within the mammary gland, insuring drug's action against the organism and increasing the chances of microbiological cure.

Blitz therapy presents overall very good results for eradicating *S. agalactiae* in positive herds; however, this practice should not be seen as the only solution to prevent mastitis but as an auxiliary method to integrated programs of mastitis control (GREEN, 2000). Teat disinfection after milking and dry cow therapy was implemented simultaneously to treatment during lactation. It is also important to adopt hygienic practices such as pre and post dip, drying teats with disposable paper towels, adequate milking equipment maintenance and cleaning, and dry cow therapy, if not earlier, at least simultaneously to implementation of blitz therapy.

In this study, treatment of cows carrying *S. agalactiae* resulted in significant reduction of SCC in bulk tank. Similar results were seen in a study conducted by Goldberg et al. (1991) in a herd with SCC average of 918,000 cells/ml before blitz therapy; it was reduced to 439,000 cells/ml 30 days after treatment. Before treatment, 40 animals positive for *S. agalactiae* were responsible for 47.04% of SCC in bulk tank milk; this value was reduced to 18.48% after removing *S. agalactiae*, proving the role of this agent in the high incidence of SCC in the herd.

Even though the impact of *S. agalactiae* in milk production is well known, a high variability of the effects of blitz therapy on milk production can be seen. Studies on the effect of blitz therapy in milk production indicate great benefits of this treatment to animals in early lactation. Yagamata et al. (1987) obtained 98% of cure rate for *S. agalactiae* and demonstrated a better cost-benefit for treated cows in early and mid-lactation, with earnings of US\$ 396 and US\$ 237, respectively. For treated cows in late lactation, the benefit was comparatively lower, US\$ 55. However, these authors did not consider the impact of these infected animals by contagious agents in the new intramammary infections in the herd, since they are the main source of infection in healthy animals (TYLER et al., 1992; EDMONDSON, 2011). Reduction of clinical mastitis and non-intentional culling of animals, increase of milk production in next lactation and reduction of SCC in bulk tank should be taken into consideration while deciding whether to perform the therapy, mainly in programs with payment for quality.

Mastitis control programs using milking hygiene practices and good herd management to reduce the prevalence of *S. agalactiae* may be more economically feasible than the blitz therapy, but in cases when these programs are not effective in reducing the prevalence of *S. agalactiae*, treatment during lactation is reasonable (EDMONDSON, 2011). Given the reduction in percentage of SCC in bulk milk tank of treated cows, it is possible to evaluate the impact of *S. agalactiae* in milk quality. Blitz therapy should be considered in cases in which industries' penalties regarding high SCC are economically representative, when the prevalence of infected animals and losses related to milk production are high, as well as in institutional programs, either public or private, of SCC control in specific regions.

Farms undertaking eradication of *S. agalactiae* need to monitor the progress of the control, especially in 'clean' cows groups, which have been segregated or treated, in order to get early warning of re-emerging problems. New

infection rates, provided by individual somatic cell counts, are key in monitoring the efficacy of those treatments.

### CONCLUSION

Use of Blitz Therapy to control subclinical mastitis caused by *S. agalactiae* proved to be technically feasible, resulting in microbiological cure of cows and reduction of SCC at days 14 and 21 post treatment. Use of Blitz Therapy to control subclinical mastitis caused by *S. agalactiae* reduced the somatic cell count of bulk tank.

### ACKNOWLEDGMENTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig), Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

### REFERENCES

ALVES, E. D. C.; DAHMER, A. M.; BORGES, A. F. Total bacterial count and somatic cell count in refrigerated raw milk stored in communal tanks. **Brazilian Journal of Food Technology**, v. 17, n. 3, p. 221-225, 2014.

BRAZIL. MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. INSTRUÇÃO NORMATIVA n. 62, de 29 de dezembro de 2011. **Regulamento Técnico de Produção, Identidade e Qualidade do Leite tipo A, o Regulamento Técnico de Identidade e Qualidade de Leite Cru Refrigerado, o Regulamento Técnico de Identidade e Qualidade de Leite Pasteurizado e o Regulamento Técnico da Coleta de Leite Cru Refrigerado e seu Transporte a Granel**. Diário Oficial da União, Brasília, 30 dez. 2011. Seção 1.

BRITO, J. R. F. **Coleta de amostra de leite para determinação da composição química e contagem de células somáticas**. Juiz de Fora: Embrapa Gado de Leite. (Embrapa Gado de Leite. Circular técnica, 62), 16 p., 2001.

BRITO, M. A. V. P.; BRITO, J. R. F. **Diagnóstico microbiológico da mastite**. Juiz de Fora, MG: Embrapa Gado de Leite, (Embrapa Gado de Leite. Circular Técnica, 55), 26 p., 1999.

EDMONDSON, P. Blitz therapy for the eradication of *Streptococcus agalactiae* infections in dairy cattle. **In Practice**, v. 33, p. 33-37, 2011.

ERSKINE, R. J.; EBERHART, R. J. Herd benefit-to-cost ratio and effects of a bovine mastitis control program that includes blitz treatment of

*Streptococcus agalactiae*. **Journal of the American Veterinary Medical Association**, v. 196, n. 8, p. 1230-1235, 1990.

GOLDBERG, J. J. et al. An update survey of bulk tank milk quality in Vermont. **Journal Food Protection**, v. 54, n. 7, p. 549-553, 1991.

GOODGER, W. J. et al. Using simulation modeling to assess the benefit of lactational therapy for subclinical *Streptococcus agalactiae* mastitis. **Acta Veterinaria Scandinavica Supplements**, v. 84, p. 377-80, 1988.

GREEN, M. Treating bovine mastitis. **Cattle Practice**, v. 8, p. 183-184, 2000.

HARMON, R.J. et al. **Microbiological procedures for the diagnosis of bovine udder infection**. Arlington: National Mastitis Council, 1990. 34p

KEEFE, G. Update on Control of Staphylococcus aureus and Streptococcus agalactiae for management of mastitis. **Veterinary Clinics of North America: food animal practice**, v. 28, p. 203-216, 2012.

KLEI, L.; YUN, J. Effects of milk somatic cell count on cottage cheese yield and quality. **Journal of Dairy Science**, v. 81, p. 1205-1213, 1998.

LITTELL, R.C. et al. **SAS for Mixed Models**, 2nd Edition. SAS Institute Inc. Cary NC. 2006.

MILLIKEN, G.A.; JOHNSON, D.E. **Analysis of Messy Data, Vol. 1: Designed Experiments**, 2nd Edition. Chapman & Hall/CRC, New York. 2009.

R CORE TEAM R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>. 213.

REYES, J. M. et al. Evaluation of the efficacy of intramuscular versus intramammary treatment of subclinical *Streptococcus agalactiae* mastitis in dairy cows in Colombia. **Journal of dairy science**, v. 98, p. 5294-5303, 2015.

SAS INSTITUTE INC. SAS/STAT® **User's guide: Statistics**, Version 9.2. SAS Institute, Cary, US. 2009.

SMITH, A. R.; WARD, G. E. Evaluation of methods for control of *Streptococcus agalactiae* in dairy herds. **The Canadian Veterinary Journal**, v. 16, p. 109-113, 1975.

TYLER, J. W.; WILSON, R. C.; DOWLING, P. Treatment of subclinical mastitis. **Veterinary Clinics of North America: Food Animal Practice**, v. 8, p. 17-27, 1992.

VIANA, F.A.B. **Fundamentos da Terapêutica Veterinária**. 2000. Belo Horizonte: Escola de Veterinária da UFMG, ed 2000.

YAGAMATA, M. et al. The economic benefit of treating subclinical *Streptococcus agalactiae* mastitis in lactating cows. **Journal of American Veterinary Medical Association**, v. 191 n. 12 p. 1556-1560, 1987.