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Original Article
Livestock Diseases

Efficacy of antimicrobial therapy in association with vaccination on the bacteriological cure of subclinical mastitis caused by *Staphylococcus aureus* in lactating cows[1]

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ABSTRACT.- Garcia B.L.N., Pinheiro E.S.C., Fidelis C.E., Freu G., Leite R.F., Moreno A.M. & Santos M.V. 2022. **Efficacy of antimicrobial therapy in association with vaccination on the bacteriological cure of subclinical mastitis caused by *Staphylococcus aureus* in lactating cows**. *Pesquisa Veterinária Brasileira 42:e07064, 2022*. Departamento de Nutrição e Produção Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Rua Duque de Caxias 225, Jardim Elite, Pirassununga, SP 13635-900, Brazil. E-mail: mveiga@usp.br

The objective of this study was to evaluate the efficacy of the antibiotic therapy associated with vaccination on the microbiological cure rate of subclinical mastitis caused by *Staphylococcus aureus* in lactating dairy cows. A total of five herds, from which 72 cows (120 mammary quarters - MQ) were diagnosed with *S. aureus* subclinical mastitis, were included in this study. Cows were randomly allocated to one of three treatment groups: a) Control (no treatment); b) ATB (antibiotic therapy); and c) ATB+VAC (antibiotic therapy plus vaccination against S. aureus). Intramammary treatment consisted of twice-daily infusion of ampicillin 75mg + cloxacillin 200mg, for 5 days. Parenteral treatment was done by injection of a single dose (7.5mg/kg) of enrofloxacin, on the first day of the treatment protocol. Vaccinated cows received three doses of a commercial vaccine 14 days before treatment (d-14), on the first day of treatment protocol (d1), and 14 days after the treatment protocol (d+14). Non-treated cows had a lower cure rate (0.06) than cows treated with ATB (0.84) and ATB+VAC (0.85). No difference in cure rate was observed between cows treated with ATB and ATB+VAC. On the other hand, vaccinated cows had lower somatic cell count (SCC) after 28 days of the treatment protocols (4.76 $\log_{10}$) than non-treated cows (5.37 $\log_{10}$). In conclusion, treatment with intramammary ampicillin and cloxacillin, associated with intramuscular enrofloxacin presented a high cure rate for SCM caused by *S. aureus* during lactation. The use of vaccination against *S. aureus* in association with antibiotic therapy did not increase the cure rate of MQ during lactation, but it was effective in reducing the SCC when compared to non-treated MQ. Although to ensure that the decrease of the SCC in ATB+VAC group was associated with the vaccination, the study should have included an additional group of only vaccinated cows, without antimicrobial therapy, with was not done in the present study, and therefore is one of the limitations of the experimental protocol used.

INDEX TERMS: Antimicrobial therapy, vaccination, bacteriological cure, subclinical mastitis, *Staphylococcus aureus*, lactation, cows, bovine, intramammary infection.

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RESUMO.- [Eficácia da terapia antimicrobiana associada à vacinação na cura bacteriológica de mastite subclínica causada por *Staphylococcus aureus* em vacas em lactação.] O objetivo deste estudo foi avaliar a eficácia da antibioticoterapia associada à vacinação sobre a taxa de cura microbiológica

de mastite subclínica causada por *Staphylococcus aureus* em vacas leiteiras em lactação. Foram selecionados 5 rebanhos, dos quais 72 vacas (120 quartos mamários, QM) foram diagnosticadas com mastite subclínica por *S. aureus* e foram alocadas aleatoriamente em um de três grupos de tratamento: a) Controle (sem tratamento); b) ATB (antibioticoterapia); e c) ATB+VAC (antibioticoterapia mais vacinação contra *S. aureus*). O tratamento intramamário consistiu em infusão de ampicilina 75 mg + cloxacilina 200 mg duas vezes ao dia, durante 5 dias. O tratamento parenteral foi feito por injeção de uma dose única (7,5 mg/kg) de enrofloxacina, no primeiro dia do protocolo de tratamento. As vacas vacinadas receberam três doses de uma vacina comercial, 14 dias antes do tratamento (d-14), no primeiro dia do protocolo de tratamento (d1) e 14 dias após o protocolo de tratamento (d+14). A taxa de cura das vacas não tratadas foi menor (0,06) do que das vacas tratadas com ATB (0,84) e ATB+VAC (0,85). Não foi observada diferença de taxa de cura entre vacas tratadas com ATB e ATB+VAC. Por outro lado, as vacas vacinadas apresentaram menor a contagem de células somáticas (CCS) após 28 dias de tratamento (4,76 $\log_{10}$) do que em vacas não tratadas (5,37 $\log_{10}$). Em conclusão, o tratamento com ampicilina e cloxacilina intramamária, associados à enrofloxacina intramuscular, apresentou alta taxa de cura para MSC causada por *S. aureus* durante a lactação. A utilização da vacinação contra *S. aureus* associada à antibioticoterapia não aumentou a taxa de cura dos QM durante a lactação, mas foi eficaz na redução do CCS quando comparada à QM não tratados. Entretanto, para ter certeza que a diminuição da CCS no grupo ATB+VAC estivesse associada à vacinação, o estudo deveria ter incluído um grupo adicional de apenas vacas vacinadas, sem terapia antimicrobiana, o que não foi feito no presente estudo e, portanto, é um das limitações do protocolo experimental utilizado.

TERMOS DE INDEXAÇÃO: Terapia antimicrobiana, vacinação, cura bacteriológica, mastite subclínica, *Staphylococcus aureus*, vacas, lactação, infecção intramamária.

INTRODUCTION

Bovine mastitis is the main disease of dairy herds, which reduces milk yield and milk quality (Gonçalves et al. 2020). *Staphylococcus aureus* is a contagious pathogen frequently isolated from subclinical (SCM) and chronic cases of mastitis around the world (Keefe 2012). Dairy cows infected by *S. aureus* are an important reservoir of this pathogen in dairy herds, which can be transmitted from cow to cow during milking (Keefe 2012, Pumipuntu et al. 2017), and that presents a low cure rate to antimicrobial therapy during lactation (Keefe 2012).

The efficacy of antimicrobial therapy against *S. aureus* is influenced by mastitis severity, and factors related to cows, such as age, days in milk (DIM), and the number of infected quarters (Reksen et al. 2006). Moreover, *S. aureus* presents some characteristics that hinder antimicrobial activity, as the ability to survive within phagocytes, biofilm production, high genetic variability among strains, and antimicrobial resistance (Cheng & Han 2020, Ren et al. 2020). The duration of the antimicrobial protocols used for treating mastitis caused by *S. aureus* can affect the cure rate (Deluyker et al. 2005, Barkema et al. 2006). For example, short-duration treatment commonly used in cases of intramammary infections (IMI) during lactation can result in a low cure rate (Barkema et al. 2006), while extended therapy (e.g., 5 to 8 days) results in higher cure rate against *S. aureus* causing mastitis (Oliver et al. 2004, Roy & Keefe 2012).

Although in the USA, the FDA (Food and Drug Administration) prohibits the use of fluoroquinolones in food-producing animals since 1997 (Matushek 2013), in Brazil, fluoroquinolones (e.g., enrofloxacin) is approved for systemic treatment of mastitis (Tomazi & Dos Santos 2020). Enrofloxacin is characterized by high tissue perfusion, long half-life in blood, and its metabolite (ciprofloxacin) is maintained in high concentrations in both blood and milk (Rantala et al. 2002). Due to these characteristics, parenteral administration of enrofloxacin in association with extended intramammary antibiotic therapy may be an alternative to increase the cure rates of SCM caused by *S. aureus* during lactation.

Furthermore, vaccination against *S. aureus*, combined with extended therapy, can be used as a strategy in *S. aureus* control and to improve the cure rate during lactation (Luby & Middleton 2005, Smith et al. 2006). Vaccination could increase the capacity of the cow's immune system to fight *S. aureus* IMI and therefore could increase the efficiency of antimicrobial treatments. A previous study reported a cure rate of 0.66 of vaccination associated with extended therapy with pirlimycin for mastitis caused by *S. aureus* in lactating dairy cows (Luby & Middleton 2005).

The efficacy of extended intramammary therapy in association with parenteral enrofloxacin on the cure rate of SCM caused by *S. aureus* has not yet been evaluated. Studies evaluating protocols for the treatment of SCM caused by *S. aureus* could be helpful as additional control measures of this pathogen in dairy herds with the prevalence of this pathogen. Therefore, the present study aimed to evaluate the efficacy of combined antibiotic therapy (intramammary + systemic) associated or not with vaccination for the treatment of SCM caused by *S. aureus* during lactation.

MATERIALS AND METHODS

Herd selection, cow's enrollment and sample collection. Five dairy herds located in Southeast Brazil [Minas Gerais (n=3) and São Paulo (n=2) States] that presented the history of SCM caused by *Staphylococcus aureus* (15% of the cows infected) were selected for this study. Dairy herds had an average of 190 (ranging from 88 to 312) Girolando crossbred (*Bos taurus* × *Bos taurus indicus*) dairy cows, which were housed in paddocks and had an average daily milk production of 22kg per cow.

Before the start of this study, all lactating cows from each herd were evaluated for the occurrence of *S. aureus* IMI. For this, composite milk samples were collected for SCC analyses. Cows with SCC>200.000 cells/mL, had three composite milk samples (pool of all mammary quarters - MQ), with a one-week interval between samplings. Samples were aseptically collected for microbiological culture. Microbiological culture analyses were performed per the National Mastitis Council Guidelines (NMC) (Adkins et al. 2017).

A total of 117 dairy cows were diagnosed with IMI caused by *S. aureus* and were initially included in the present study. However, cows that required therapeutic interventions (n=15), treated with modified therapy protocols (n=4), involuntary culling from dairy herds (n=18), and early drying off (n=8) were excluded. After exclusions, a total of 72 dairy cows were used for treatment allocation in the present study.

Microbiological identification and somatic cell count. Dairy cows (n=72) previously identified with *S. aureus* SCM based on composite samples had MQ milk samples collected for microbiological identification and SCC analysis. Milk samples for *S. aureus* identification were collected and submitted to the microbiological culture following NMC Guidelines (Adkins et al. 2017). In addition, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed to confirm *S. aureus* at the species level. The MALDI-TOF MS analysis was performed according to Barcelos et al. (2019), and species identification level was considered when MALDI score ≥2.00.

SCC was performed in MQ milk samples collected on day one and 28 days after treatment. Milk samples were collected into a plastic tube containing 2-Bromo-2-nitropropane-1,3-diol chemical preservative (Bronopol, Microtabs II, D&F 131 Control Systems Inc., Norwood/MA, USA) and analyzed by flow cytometry using the Somacount 300® equipment (Bentley Instruments Inc., 133 Chaska/MN, USA).

Mastitis treatment protocols and bacteriological cure evaluation. Selected cows (n=72, totaling 120 infected MQ) were allocated according to DIM and parity and randomly distributed to one of the three treatments: 1) Control – no treatment (n=38 MQ, n=22 cows); 2) ATB (antibiotic therapy; intramammary and parenteral antimicrobial therapy; n=44 MQ, n=31 cows); and 3) ATB+VAC (intramammary antimicrobial plus parenteral antimicrobial therapy plus vaccination against *S. aureus*; n=38 MQ, n=19 cows). Intramammary treatment was performed with an infusion of ampicillin (75 mg) + cloxacillin (200mg) (Bovigam L, Bayer Animal Health, North Ireland), twice/d, for 5 days. Parenteral treatment was performed by a single intramuscular administration of enrofloxacin (7,5mg/kg) (Kinetomax, Bayer Animal Health, Brazil) on the first day of treatment. For vaccination, cows received three doses of a commercial vaccine (TopVac, Hipra, Spain) 14 days before treatment (d-14), on the first day of treatment (d1), and 14 days (d+14) after treatment (Fig.1).

For bacteriological cure evaluation, quarter milk samples were collected at 14, 21, and 28 days after the onset of mastitis treatment (Fig.1). MQ was considered cured if none of the milk samples after treatment (n=3) had *S. aureus* isolation (Sears et al. 1990). On the other hand, MQs with at least one positive result for *S. aureus* were considered not cured (Sol et al. 1997, Deluyker et al. 2005).

Data analysis. Bacteriological cure rates were evaluated considering MQ as the experimental unit. Data were analyzed using multivariate logistic regression, using the PROC GLIMMIX of SAS version 9.3 (SAS Institute, Cary/NC, USA), to assess the effect of independent variables (parity, DIM, number, and position of infected quarters per cow). Herd and cow were included in the model as a random effect. Univariate analyses were used to evaluate the independent variables, and a manual selection and elimination procedure was performed in which only those variables with $P \leq 0.30$ were maintained in a multivariable model (Martins et al. 2019). The final model used was as follows:

$$\text{logit}(\text{pi}) = \beta_0 + \beta_1 \times \text{Treat} + \beta_2 \times \text{SCCLog} + \text{Cow}(\text{random}) + \text{Herd}(\text{random}) + \text{e}$$

Where: logit(pi) = logistic function of the cure rate; β0 = intercept; β1 = regression coefficient for the treatment protocols; β2 = regression coefficient for SCC before treatment, converted into a logarithmic scale ($\log_{10}$); Cow (random) = cow random effect; Herd (random) = herd random effect; and e = residual error. The binary distribution with the logistic function was used and a first-order autoregressive correlation structure was used for the best fit of the model. SCC was converted into a logarithmic scale ($\log_{10}$) and evaluated considering the MQ as the experimental unit. An analysis of repeated measures over time was performed by a general linear mixed model, using the SAS version 9.3 PROC MIXED (SAS Institute, Cary/NC, USA) to verify the effect of the independent variables: herd, treatment,

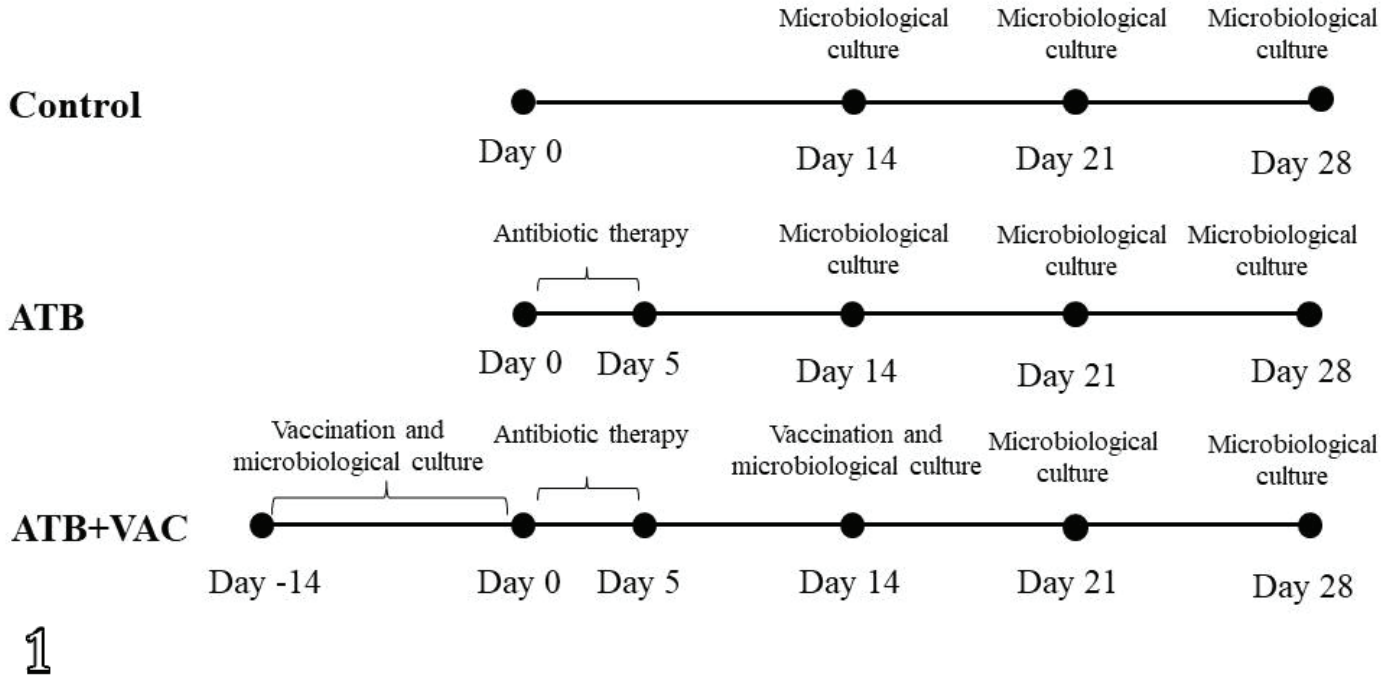

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Fig.1. Milk sampling according to treatment protocols of cows with subclinical mastitis caused by *Staphylococcus aureus*.

parity, number, and position of infected quarters per cow, time and interaction time x treatment and the DIM covariate on the dependent variable. The general linear mixed model was:

$$Y = \mu + Treat + Herd + Parity + DIM + IQ + QP + error$$

Where: Y = delta log(10) of MQ SCC (last SCC log - 1st collection); μ = overall mean; Treat = fixed treatment effect (Control, ATB and ATB+VAC); Herd = random herd effect; Parity = fixed effect of the parity (1 = primiparous; 2 = multiparous); DIM = effect of the covariate days in lactation; IQ = infected quarters - fixed effect of the number of quarters infected per cow (1 = only one MQ infected with *S. aureus*, 2 = two or more MQ infected with *S. aureus*); QP = MQ position - fixed effect of the position of the infected MQ(s) (Front = left and right front MQ; Rear = left and right rear MQ); error = random error associated with each observation of the main plot and subplot. For all analyses, values of $P \leq 0.05$ were considered significant.

RESULTS

Treated cows and bacteriological cure

A total of 72 cows (120 MQ) diagnosed with *Staphylococcus aureus* SCM were evaluated in this study. From these 120 infected MQ included, 11 (0.09) were from cows in the early lactation (0-100 DIM); 35 (0.30) in the middle of lactation (>100-200 DIM), and 73 (0.61) were in the final third of lactation (>200 DIM; Table 1). On average, each cow treated during lactation had 1.7 MQ infected with *S. aureus*. MQs from cows with two or more infected MQ accounted for 82 (0.68) while 38 (0.32) were from cows with only one quarter infected (Table 1).

Mammary quarters treated with ATB and ATB+VAC had a higher bacteriological cure rate (0.84 and 0.85, respectively; P=0.002) than non-treated MQ (0.06). However, there was no difference in cure rate between ATB and ATB+VAC treatments (Table 2). There was no effect of the SCC log before treatment on the bacteriological cure rates (P=0.10).

Somatic cell count

Data of SCC from 85 MQ were available and were analyzed. The SCC means before and after the lactation treatment protocols are shown in Table 3. There was an interaction between time and treatments (P=0.04) on the SCC. On the first day of antibiotic therapy there was no difference between the SCC of the 3 treatments, however, after 28 days, the SCC of the MQ treated with ATB+VAC (4.76 $\log_{10}$) was lower than control MQ (5.37 $\log_{10}$).

DISCUSSION

Staphylococcus aureus is one of the main mastitis-causing pathogens, and this IMI presents a low cure rate to antimicrobial therapy during lactation (Keefe 2012). This study evaluated the efficacy of antibiotic therapy associated with vaccination against *S. aureus* during lactation on bacteriological cure rate.

Our results showed that bacteriological cure rate was on average 0.84 for ATB and ATB+VAC treatments. Cure rates observed in our study were higher than those described in previous studies of extended therapy during lactation (5 to 8 days of treatment) (Oliver et al. 2004, Roy et al. 2009). Treatment with intramammary cephapirin for 5 days resulted in a cure rate of 0.26 (8/31 cows) (Roy et al. 2009), while that treatment for 5 days with intramammary ceftiofur resulted

Table 1. Descriptive results of cure rates of *Staphylococcus aureus* treatments during lactation, according to cows' characteristics, number of infected quarters and position of infected quarters (120 MQs from 72 cows)

Variable		MQ (n, proportion)	Cure rate* (n, proportion)
Treatment**	Control	38 (0.32)	1 (0.03)
	ATB	44 (0.37)	34 (0.77)
	ATB + VAC	38 (0.32)	32 (0.84)
Parity	1	21 (0.18)	16 (0.78)
	≥2	99 (0.83)	51 (0.52)
DIM	1	11 (0.09)	5 (0.46)
	2	36 (0.30)	18 (0.50)
	3	73 (0.61)	44 (0.60)
NIQ	1	38 (0.32)	23 (0.61)
	≥2	82 (0.68)	44 (0.52)
QP	Rear	63 (0.53)	35 (0.56)
	Front	57 (0.48)	32 (0.56)

MQ = mammary quarters; * Number of cured cows after treatment of *S. aureus* and cure rates in percentage; ** Control = no treatment, ATB = antibiotic therapy, ATB + VAC = antibiotic therapy + vaccination; DIM = days in milk class: class 1 = 1 to 100 days in milk, class 2 = >100 to 200 days in milk, class 3 = >200 days in milk; NIQ = number of mammary quarters infected with *S. aureus*; QP = infected mammary quarter position.

Table 2. Results of the mixed logistic regression model regarding the adjusted bacteriological cure risk of mammary quarters infected by *Staphylococcus aureus* and submitted to treatment during lactation

Variable	Coefficient	SE	LSM	SEM	CI 95%		*P*-value
Bacteriological cure							
Intercept	4.99	2.17					
Treatment*							0.0029
ATB + VAC	Ref.		0.85[a]	0.07			
ATB	-0.05	0.86	0.84[a]	0.09	0.16	5.47	
Control	-4.50	1.26	0.06[b]	0.06	<0.001	0.15	
SCC log**	-1.26	0.75			0.06	1.31	0.1036

SE = standard error, LSM = least square mean, SEM = standard error of mean, CI = confidence interval; * Control = no treatment, ATB = antibiotic therapy, ATB + VAC = antibiotic therapy + vaccination; ** SCC log = somatic cell count, converted into a logarithmic scale ($\log_{10}$); [a,b] Different letters represent statistical difference (P<0.05) between treatment groups means.

Table 3. Results of SCC ($\log_{10}$) linear mixed models of mammary quarters according sampling periods (d1 or d28) by treatment groups

Sampling day	Treatment			SE	*P*-value		
	Control	ATB	ATB+VAC		Treat.	Time	Treat. x Time
1	5.76[a]	5.46[a]	5.61[a]	0.059	0.17	<0.001	0.04
28	5.37[a]	5.03[ab]	4.76[b]	0.097			

SCC = somatic cell count, Control = without antibiotic therapy and vaccination, ATB = antibiotic therapy, ATB+VAC = antibiotic therapy plus vaccine, SE = standard error; [a,b] Different letters represent statistical difference (P<0.05) between treatment groups.

in a cure rate of 0.17 (2/12 MQ) and 0.36 (4/11 MQ) with 8 days of treatment (Oliver et al. 2004). On the other hand, Deluyker et al. (2005) reported that MQs (n = 53) treated during 8 days with intramammary pirlimycin presented a bacteriological cure rate of 0.86, which were similar to the results observed in the present study. However, direct comparisons between studies are limited due to different definitions for a bacteriological cure, cows' characteristics, frequency of infected MQs, therapy protocols (short *vs*. extended), and type of antimicrobial used. According to Roy & Keefe (2012), extended intramammary therapy for 5-8 days was the most efficient therapeutic option to treat mastitis caused by *S. aureus* during lactation. This aforementioned strategy was used in the present study.

Also, in the present study, a cure rate was assessed based on three samples from all treated MQs up to 28 days after treatment, to minimize the number of false-negative results. Furthermore, MQ was considered cured with only three negative results for *S. aureus* after treatment, whereas other studies considered that two negative results for *S. aureus* after treatment (21 and 28 days after treatment) was sufficient for considering the MQ as cured (Sears et al. 1990, Molina et al. 2018).

Another reason for the high cure rate in treatment during lactation may be the use of injectable enrofloxacin. This antimicrobial is a fluoroquinolone with low toxicity and high tissue penetration (Suojala et al. 2010, Attili et al. 2016). and that presents bioavailability and long half-life in blood (Lizondo et al. 1997). In previous studies *in vitro*, enrofloxacin demonstrated antimicrobial activity in the presence of milk (Fang & Pyörälä 1996), and the class of fluoroquinolones (to which enrofloxacin belongs) may have a positive effect on the modulation of the immune system, by increasing the ability of neutrophils of phagocytosis (Hoeben et al. 1997). Furthermore, when enrofloxacin is distributed in the bloodstream of the cow, it is degraded, giving rise to a metabolite (ciprofloxacin), which also has a bactericidal action (Suojala et al. 2010). After systemic administration of enrofloxacin, its metabolite ciprofloxacin can be found in high concentrations in blood and milk (Rantala et al. 2002). It is important to note that, although in the USA, the use of fluoroquinolones in food-producing animals was prohibited (Matushek 2013), in Brazil, fluoroquinolone use is still approved for systemic treatment of mastitis (Tomazi & Dos Santos 2020).

In addition to the type of antimicrobial therapy administered during lactation, factors related to cows, as age, DIM, and number of infected quarters (Reksen et al. 2006) might also have affected the efficacy of antimicrobial therapy against *S. aureus.* In our study, none of these variables affected bacteriological cures and were excluded from the final model. However, non-adjusted bacteriological cure rates were higher to MQ of primiparous (0.78) cows than MQ of multiparous (0.52) cows. This is consistent with results reported in other studies (Sol et al. 1997, Taponen et al. 2003, Deluyker et al. 2005). The higher cure rate of primiparous cows may have occurred because 1) older cows may have chronic infections for a longer time than infections in younger cows, and increasing the duration of IMI reduces the cure rate (Sol et al. 1994, 1997, Dingwell et al. 2003); 2) the cow's immune system becomes less effective with increasing age (Sol et al. 1997); 3) the larger size of the mammary gland of adult cows can contribute to reducing the chances of cure, as the antibiotic must diffuse and eliminate *S. aureus* in a larger volume of tissue (Barkema et al. 2006); 4) SCC of the MQ of primiparous cows was lower than the MQ of multiparous cows, before treatment; and MQs with lower SCC had a greater cure rate than MQs with higher SCC (Sol et al. 1997). Regardless of the treatment group, in our study, the SCC of MQ from primiparous cows was lower than multiparous cows. In previous studies, high SCC reduced the cure rate for clinical (Sol et al. 2000) and subclinical (Sol et al. 1997) mastitis caused by *S. aureus*.

In our study, the lactation stage did not affect the cure rate and was eliminated from the final model. In contrast to our results, Sol et al. (1997) and Deluyker et al. (2005) reported that the cure of cows at the end of lactation (>200 DIM) was higher than the cure of cows at the beginning of lactation (<100 DIM). This effect can be attributed to the more rapid antimicrobial elimination due to the greater milk production in early lactation (Deluyker et al. 2005). Considering that there was no effect of the lactation stage on the cure rate in the present study, the results suggest that the treatment of cows at the beginning of lactation could improve the profits of milk production, because these cows would remain healthy for a longer period than if they were treated at the end of lactation.

MQs from cows with only one quarter infected with *S. aureus* had a similar cure rate as quarters from cows with two or more MQ infected. The results diverge from previous studies that observed that cows with two or more quarters infected by *S. aureus* had a lower cure rate than cows with only one quarter infected (Sol et al. 1994, Osterås et al. 1999). The position of the MQ also did not affect the cure rate. In other studies, hindquarters had a lower cure rate than forequarters (Sol et al. 1997, Deluyker et al. 2005). Barkema et al. (2006) described that the lower cure rate of hindquarters may be due to the greater volume of hindquarters compared to forequarters, which could accelerate antibiotic elimination, and could be a reason to decrease the risk of cure.

Our results showed the effect of the interaction between treatment and vaccination on the SCC of the MQ. After antibiotic therapy, the SCC of the MQ treated with ATB+VAC was lower than non-treated MQs. According to Schukken et al. (2014), who evaluated the same vaccine used in this study, vaccination decreased the proliferation rate of *S. aureus* by 0.45. In the present study, the immune response of vaccinated cow may have reduced the proliferation rate of *S. aureus,* which resulted in lower SCC. Although to ensure that the decrease of the SCC in ATB+VAC group was associated with the vaccination, the study should have included an additional group of vaccinated only cows, without antimicrobial therapy, with was not done in the present study, and therefore is one of the limitations of the experimental protocol used.

After the administration of the second dose of the vaccine protocol (at the start of antibiotic therapy), MQ SCC means was 5.83 $\log_{10}$ cells/mL in vaccinated cows. By day 28 after the start of antibiotic therapy, the mean of MQ SCC of the cows treated with ATB+VAC treatment reduced to 4.76 $\log_{10}$ cells/mL. But no effect of ATB treatment was observed in the SCC. This result suggested that the reduction in SCC from the ATB+VAC treated cows was influenced by vaccination. The findings can be attributed to possible protection against *S. aureus* IMI developed by vaccination, although this result diverges from Middleton et al. (2009) who did not observe

a difference between the vaccinated and the control group in the SCC.

One limitation of the present study is that to evaluate whether cows had a persistent infection or a new IMI, it would be necessary to use molecular characterization to confirm that the same strain of *S. aureus* was isolated before and after treatment. Another limitation of this study is the absence of an intramammary antimicrobial treatment group. In this case, it would be possible to evaluate the effect of the use of intramammary antimicrobial from the systemic treatment. This could help the selection of the best therapeutic strategy against *S. aureus* during lactation, in addition to reducing the overuse of antimicrobials in dairy herds, especially fluoroquinolones that have been prohibited as treatment in food-producing animals in other countries (Matushek 2013).

CONCLUSION

Treatment with intramammary ampicillin and cloxacillin, associated with intramuscular enrofloxacin presented a high cure rate for subclinical cases of mastitis (SCM) caused by *Staphylococcus aureus* during lactation. The use of vaccination against *S. aureus* in association with antibiotic therapy did not increase the cure rate of mammary quarters (MQ) during lactation, but it was effective in reducing the somatic cell count (SCC) when compared to non-treated MQ.

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Conflict of interest statement.- The authors declare no conflict of interest.

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