

Prevalence reduction of *Streptococcus agalactiae* by intramammary antibiotic treatment in a Holstein dairy herd under tropical conditions

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Introduction

Streptococcus agalactiae and *Staphylococcus aureus* are contagious pathogens and are considered to be the major mastitis pathogens, because of their large effect on milk quality, production, and cow somatic cell count (SCC).¹ The primary method to spread these pathogens is cow to cow, so the focus of prevention should be to reduce or eliminate herd infection.¹ In herds where the pattern of subclinical mastitis infection is by these contagious pathogens, the eradication of *S. agalactiae* and control and prevention of *S. aureus* must be the objectives. For *S. agalactiae*, eradication can be completed rapidly through a culture and treatment program with minimal culling. For *S. aureus*, treatment success, particularly during lactation, is often disappointing and depends on cow, pathogen, and treatment factors.¹ This way, the culling of animals with chronic infection by *S. aureus* is the most indicated strategy. Therefore, the microbiological diagnosis of mastitis is fundamental for the adoption of specific control measures, segregation and culling of animals with chronic infection, evaluation of treatment efficacy and establishment of antimicrobial susceptibility patterns.² The objective of this study was to assess the treatment with intramammary antibiotic on a Holstein dairy herd in the process of *S. aureus* and *S. agalactiae* control under tropical conditions.

Material and methods

The study was conducted in a dairy cattle herd located in the Zona da Mata, Minas Gerais, Brazil. The herd belongs to the Holstein Cattle Association of Minas Gerais State, which has 112 herds. These herds have some common characteristics that are: breed, nutritional and reproductive management, milking machine and keep records on the productive, economic and mammary gland health indices. The studied herd was composed of Holstein cows, with an average of 142 lactating cows/month and a mean production of 8,050 kg of milk over 305 days of lactation. The lactating cows were subjected to official milk production control through a monthly assessment of milk production and collection of milk samples to determine the composition (fat, protein, lactose and total solids) and somatic cells count. Milk samples from the herd (bulk tank) were collected directly into vials containing preservative (Bronopol®, D&F Control Systems Inc., USA) for somatic cell counting (SCC). SCC was performed in the Milk Quality Laboratory of Embrapa Dairy Cattle in an automated flow cytometer (Somacount 300, Bentley Instruments Inc., USA).³

During the period from January 2012 to January 2013, milk samples were collected from individual lactating cows for microbiological tests. These samples were collected during the months of January (n = 159), February (n = 144), March (n = 131), April (n = 146), May (n = 157), August (n = 149) and November (n = 147) in 2012 and January (n = 134) in 2013. The milk samples were taken from all lactating animals, excluding those who presented clinical mastitis at the time of collection or were being treated with antibiotics. The sampling of only lactating cows was in order to identify those infected by *S. agalactiae* for the treatment of all mammary quarters with intramammary antibiotics, as well as to identify chronically infected lactating cows by *S. aureus* for possible culling. The intramammary antibiotic



used in cows infected by *S. agalactiae* were cloxacillin (200 mg) and ampicillin (75 mg) (Bovigam®L, Bayer S.A., São Paulo, Brazil) for three times at 12 hours intervals. A disposal milk period of 72 hours was adopted according to the manufacturer's recommendation. Cows with more than two successive *S. aureus* isolations were selected for culling. The prevalence estimate of *S. agalactiae* and *S. aureus* among lactating cows was calculated dividing the number of cows infected by each pathogen by total number of lactating cows.

The milk sample collection and transport procedures were conducted according to the National Mastitis Council guidelines.^{4,5} The mastitis-causing agents were identified in the Milk Microbiology Laboratory of Embrapa Dairy Cattle by seeding 0.01 mL of each milk sample in Petri dishes containing agar-blood prepared with 5% defibrinated lamb blood. The dishes were incubated at 37 °C for 24 hours before the first reading was made, followed by incubation for an additional 24 hours for the second reading. The morphology and the number of isolated microorganism colonies were taken into consideration, and the results were interpreted according to the criteria proposed by the National Mastitis Council.^{4,5} Biochemical tests were performed to confirm the pathogens agents of mastitis. Based on the microbiological and the biochemical test results, cows infected with *S. agalactiae* received three intramammary applications of antibiotic.

Results and discussion

The percentages of infected cows with *S. agalactiae* in January 2012 and January 2013 were 61.6% and 2.2%, respectively (Table 1). For the same months, the percentages of cows infected with *S. aureus* were 28.3% and 19.4%, respectively (Table 1). The consequence of the treatment of cows infected with *S. agalactiae* was the reduction of approximately 60% in the prevalence of cows infected. The lower prevalence (0.7%) of *S. agalactiae* among lactating cows was observed on November 2012, due to one cow infected. The *S. agalactiae* prevalence reduction was continuous from January 2012 to March 2012. After this period, it was observed an oscillation on prevalence ranging from 0.7% to 6.0%. The intramammary antibiotic treatment of lactating cows infected by *S. agalactiae* was performed after

receiving results of microbiological tests.

The results of microbiological tests were provided about 5 to 7 days after collecting milk samples. The time between the collection of the milk samples and the results of the microbiological tests can be associated with oscillation of the prevalence of *S. agalactiae* among lactating cows because during this period,

cows infected were

not treated and were source of infection to cows without infection. The reduction of *S. agalactiae* prevalence was due to 161 intramammary antibiotic treatments of cows infected with *S. agalactiae* during lactation during the studied period. *S. agalactiae* is an obligate pathogen of the bovine mammary gland and rapid and successful eradication of this microorganism of the herd is achieved through the intramammary treatment of infected cows.¹ A study on contagious pathogens of mastitis was carried out in the herds of the Holstein Cattle Association of Minas Gerais State and a prevalence of 40% was observed for *S. agalactiae* among the herds.⁶ The high prevalence of *S. agalactiae* in these herds suggests that the control measures have not been adopted to effectively.⁶ The reduction of *S. aureus* prevalence was about 9% during the period studied. The *S. aureus* prevalence presented oscillation from January

Table 1. Results of microbiological test according to mastitis contagious pathogens in one dairy herd from January 2012 to January 2013

Month/Year	Number of lactating cows	Microbiological test			
		<i>Staphylococcus aureus</i>		<i>Streptococcus agalactiae</i>	
		n	%	n	%
January 2012	159	45	28.3	98	61.6
February 2012	144	56	38.9	27	18.8
March 2012	131	39	29.8	5	3.8
April 2012	146	49	33.6	7	4.8
May 2012	157	55	35.0	6	3.8
August 2012	149	52	34.9	9	6.0
November 2012	147	45	30.6	1	0.7
January 2013	134	26	19.4	3	2.2

n = number of cows infected; % = percentage of cows infected

2012 to April 2012 and after this period, a continuous reduction. Nine cows with chronic infection by *S. aureus* were culled from January 2012 to January 2013 and this procedure can be associated to *S. aureus* prevalence reduction. The success in treatment of infected animals is more difficult, because *S. aureus* is an intracellular pathogen.¹ The culling of chronically infected cows is the main indicated strategy to control the infection by this pathogen in the herd.¹ The impact on the reduction of the SCC values of the herd from 1,175,000 cells/mL to 899,000 cells/mL was probably due to a reduction in the percentage of animals infected with both pathogens. Before intramammary antibiotic treatment, the percentages of cows with values less than 200,000 cells/mL, between 200,000 and 400,000 cells/mL, and more than 400,000 cells/mL were 85.5 %, 12.0 %, and 2.5 %, respectively. The consequence of the treatment of cows infected with *S. agalactiae* was the reduction in the percentage of cows with SCCs above 400,000 cells/mL from 67.9% to 56.5% and the increase in the percentage of cows with SCCs below 200,000 cells/mL from 21.4% to 30.6%. Well management herds should have a prevalence of chronic infections (individual cows repeatedly higher than 200,000 cells/mL) of less than 5 %, and the incidence of new intramammary infections (cows breaching a 200,000 threshold) of less than 5 % on a monthly basis¹ and percentage of cows above somatic cell count cur-off limit (200,000 cells/mL) less than 20%.⁷ According to Philpot and Nickerson (1991)⁸, cows with SCCs above 400,000 cells/mL reduce their milk production from 1,188 to 1,980 pounds (approximately 538 to 898 kg) per lactation. This information was confirmed in Brazil by Cunha *et al.* (2008).⁹ In this study, Holstein animals raised in tropical conditions had less milk production as the SCC increased. Notably, the mean milk production per cow over the 305 days of lactation increased from 17,115 to 17,725 pounds (approximately 7,763 kg to 8,040 kg) during the period considered. This increase in production may be due to a reduction in the percentage of cows infected with contagious mastitis pathogens, especially *S. agalactiae*, compared to the reduction of cows infected with *S. aureus*.

Conclusions

The intramammary antibiotic treatment of lactating cows was efficient in eliminating infection caused by *S. agalactiae* and consequently the prevalence of infected animals in the herd. However, the same efficiency of intramammary antibiotic treatment in eliminating *S. agalactiae* infection was not observed for *S. aureus*. The reduction in *S. aureus* prevalence among cows was associated with the culling of cows chronically infected by this pathogen. The intramammary antibiotic treatment of cows infected with *S. agalactiae* and the culling of cows chronically infected by *S. aureus* was associated with a reduction in the SCC of the cows and herd (bulk tank) and with an increase in milk production at 305 days of lactation.

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