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Relationship between total bacteria counts and somatic cell counts from mammary quarters infected by mastitis pathogens

Relação entre contagem total de bactérias e contagem de células somáticas de quartos mamários infectados por patógenos da mastite

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ABSTRACT

*This study was conducted to establish the relationship between somatic cell count (SCC) and bacterial shedding from mammary quarters according to mastitis pathogens. Milk samples from 638 mammary quarters were examined for mastitis pathogens, SCC and total bacterial count (TBC). The raw data of SCC and TBC were used to perform descriptive statistics. The significance of the arithmetic mean differences between SCC and TBC according to bacteriological examination results was determined by a two-tailed unpaired t-test. Pearson and Spearman´s correlations were done with logarithmic data and linear regression analyses. The geometric means of the bacteriological examination results were (cells ml^{-1}; CFU mL^{-1}): no growth (52,000; 12,000), coagulase-negative staphylococci (85,000; 17,000), **Staphylococcus aureus** (587,000; 77,000); other streptococci (432,000; 108,000) and **Streptococcus agalactiae** (1,572,000; 333,000). The Pearson and Spearman´s correlations between SCC and TBC were higher than 0.60 for all mastitis pathogens. The regression analyses slopes showed different increase in TBC with the same increase in SCC according to mastitis pathogens. The slope for S. agalactiae (0.542) was higher than that for other mastitis pathogens. The results suggest that the intensity of inflammatory process was associated with number of mastitis pathogens shedding from the mammary gland.*

Key words: *mastitis pathogens, somatic cell count, bacteria shedding*

RESUMO

*Este estudo foi realizado com objetivo de estabelecer a relação entre contagem de células somáticas (CCS) e a liberação de bactérias de quartos mamários de acordo com os patógenos da mastite. Amostras de leite de 638 quartos mamários foram examinadas para identificação dos patógenos da mastite, CCS e contagem total de bactérias (CTB). Estatísticas descritivas foram utilizadas para avaliar os dados brutos de CCS e CTB. A diferença entre médias para CCS e CTB de acordo com os resultados dos exames bacteriológicos foi avaliada pelo teste T para amostras independentes. Foram realizadas a correlação de Pearson, de Spearman e regressão linear com os dados transformados. As médias geométricas de acordo com os resultados dos exames bacteriológicos foram (células mL^{-1}; UFC mL^{-1}): sem crescimento (52.000; 12.000), estafilococos coagulase negativo (85.000; 17.000), **Staphylococcus aureus** (587.000; 77000); outros estreptococus (432.000; 108.000) e **Streptococcus agalactiae** (1.572.000; 333.000). A correlação de Pearson e Spearman entre CCS e CTB foi maior que 0,60 para todos os patógenos da mastite. O coeficiente angular das regressões lineares mostrou diferentes aumentos da CTB como o mesmo aumento da CCS de acordo com os patógenos da mastite. O coeficiente angular para o S. agalactiae (0.542) foi maior em relação aos outros patógenos da mastite. Os resultados sugerem que a intensidade do processo inflamatório foi associada com a quantidade de bactérias da mastite liberada pela glândula mamária.*

Palavras-chave: *patógenos da mastite, contagem de células somáticas, liberação de bactérias*

INTRODUCTION

Differences in somatic cell count (SCC) variations and pathological modifications of the

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mammary gland, according to mastitis pathogens, have been reported in previous studies. The major pathogens cause the greatest SCC increases (***Staphylococcus aureus***, ***Streptococcus agalactiae***, coliforms, and ***Streptococcus*** spp. other than ***S. agalactiae***), and the minor pathogens (***Corynebacterium bovis*** and coagulase-negative staphylococci) usually cause only a moderate increase in SCC (SOUZA et al., 2009). Meta-analysis showed the effects associated with intramammary infection (IMI) at the quarter level by a bacterium or a group of bacteria on SCC. This study showed that the geometric mean (GM) of SCC in bacteriologically negative quarters was 68,000 cells mL^{-1}. In cases of IMI, the SCC were 357,000, 138,000, 857,000, 1,024,000 and 547,000 cells mL^{-1} in quarters infected by ***Staphylococcus aureus***, coagulase-negative staphylococci (CNS), ***S. agalactiae***, ***Streptococcus uberis*** and ***Streptococcus dysgalactiae***, respectively (DJABRI et al., 2002).

Cows with udder infection have the potential to shed in excess of 10^7 bacteria per mL according to BRAMLEY et al. (1990). For instance, ***S. agalactiae*** is usually shed in high numbers from infected glands, with a cyclic shedding pattern being typical. Infected cows in the early stage of infection with ***S. agalactiae*** can shed up to 10^2 to 10^6 bacteria per mL (KEEFE, 1997). The number of ***S. agalactiae*** and ***S. aureus*** shed in chronic infections rises and falls in a cyclic pattern (SEARS et al., 1990).

Previous studies (SEARS et al., 1990; ZADOKS et al., 2004) showed that mastitis seldom contributed large numbers of bacteria to the raw milk bulk tank. Nevertheless, the relationship between mastitis pathogens number in bulk tank milk and the percentage of infected cows showed significant a positive correlation (ZADOKS et al., 2004; RYSANEK et al., 2005). However, there is a scarcity of studies designed to assess the bacteria-shedding pattern from infected mammary gland by different mastitis pathogens. The purpose of the present study was to evaluate the shedding of bacteria from mammary quarters according to mastitis pathogens and its relationship with SCC.

MATERIAL AND METHODS

Sampling procedure

From June 2009 through December 2009, milk samples from 638 mammary quarters without clinical mastitis were collected. These mammary quarters were from 124 cows on five dairy farms located in Minas Gerais State, Brazil. From each mammary quarter, we collected three milk samples into three different vials. Teats were washed with water and then pre-dipped with a chlorine solution (150 ppm) and dried with individual paper towels after 20-30 seconds. Beginning with the teat farthest from the milker, all four teats were scrubbed with gauze moistened with 70% alcohol. Beginining with the teat closest to the milker, one or two streams of foremilk were discarded from each teat, and, for first quarter sampling, 5 to 10 mL of milk from each quarter was collected in a separate sterile vial for bacteriological examination. The second and third milk samples of each quarter, 30 to 40 mL, were used to determine total bacterial count (TBC) and SCC, respectively. The aseptic sampling procedures for bacteriological examination are described by HARMON et al. (1990). The sampling procedures for TBC and SCC were performed according to international standard (ISO 707, 2008).

Bacteriological examination

Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (HARMON et al., 1990).

Somatic cell count and total bacterial count

SCC were performed using a Bentley Somacount 300 (CHASKA, MN) and TBC determination was performed by direct counting of the bacterial cells using a Bentley IBC (CHASKA, MN).

Statistical analyses

Descriptive statistics and percentiles were performed, using the SCC and TBC values (Table 1) according to the bacteriological examination results. The normality of the raw data (SCC and TBC) distribution was tested. To be able to work with the normal distribution data, we carried out a logarithmic transformation (with logarithm base 10). The significance of the arithmetic mean differences between the $\log_{10}$SCC (Table 2) and $\log_{10}$TBC (Table 2) values according to the bacteriological examination results was determined by a two-tailed unpaired *t*-test. Linear regression analyses were done using the $\log_{10}$TBC of each mastitis pathogen isolated plus the $\log_{10}$TBC of the milk samples with no growth in the bacteriological examination as the dependent variable (y) and using the $\log_{10}$SCC of the same mammary quarters as the independent variable (x). Thus, four linear regression analyses were performed to compare the slope among mastitis pathogens. A comparison was made between the Pearson correlation coefficients and the Spearman rank correlation coefficients. The linear regression and correlation coefficients were evaluated for statistical significance (Table 3).

Table 1 - Descriptive statistics for somatic cell counts (x 1,000 cells mL^{-1}) and total bacterial counts (x 1,000cfu mL^{-1}) from mammary quarters according to bacteriological examination results.

Statistics	---------NG---------		---------CNS---------		---------STA---------		---------STR---------		----------SAG----------	
	SCC	TBC	SCC	TBC	SCC	TBC	SCC	TBC	SCC	TBC
N	421	421	64	64	69	69	39	39	45	45
GM	52	12	85	17	587	77	432	108	1,572	333
AM	281	10	258	31	1,041	260	1,504	239	2,508	759
SD	793	256	492	59	1,138	460	2,468	452	1,792	749
P10	3	2	3	4	172	14	67	21	390	18
P25	10	6	62	8	273	23	118	48	1,151	174
ME	70	10	133	15	742	66	414	100	1,839	590
P75	227	22	215	35	1,342	269	996	189	4,411	1,028
P90	610	79	595	56	2,175	772	7,426	758	5,248	2,078

N – number of samples; GM – geometric mean; AM – aritmethic mean; SD – Standard deviation ; P10 - 10^{th} percentile; P25 - 25^{th} percentile; ME – median; P75 - 75^{th} percentile; P90 - 90^{th} percentile; NG - no growth; CNS - coagulase negative staphylococci; STA - ***Staphylococcus aureus***; STR - other streptococci; SAG - ***Streptococcus agalactiae;*** SCC – somatic cell count; TBC – total bacterial count.

RESULTS

In table 1, the descriptive statistics of SCC and TBC according to bacteriological examination results are shown. Of the 638 total mammary quarter milk samples, 421 (66.0%) demonstrated no growth in the bacteriological examination. The SCC geometric mean and median were 52,000 and 70,000 cells mL^{-1}, respectively. Mastitis pathogens isolated from mammary quarter milk samples were ***S. aureus*** (69/ 10.8%), coagulase negative staphylococci CNS (64/ 10.0%), ***S. agalactiae*** (45/7.1%) and other streptococci (39/6.1%). The higher geometric mean and median SCC observed for ***S. agalactiae*** were 1,572,000 and 1,839,000 cells mL^{-1}, respectively. ***S. aureus*** (587,000 and 742,000 cells mL^{-1}) and other streptococci (432,000 and 414,00 cells mL^{-1}) demonstrated higher values. The lower values for the descriptive statistics of the SCC according to mastitis pathogens were for CNS, with values close to being bacteriologically negative. The arithmetic mean of the log_{10}SCC (Table 3) according to the bacteriological examination results increased in this order: no growth, CNS, other streptococci, ***S. aureus*** and ***S. agalactiae***. Analysis of the arithmetic mean differences between the log_{10}SCC revealed a significant difference between bacteriological examination results ($P<0.05$). Bacteriologically negative quarters had the lower log_{10}SCC arithmetic means (1.71) and were different from CNS (1.93). The ***S. aureus*** (2.77) and other streptococci (2.64) log_{10}SCC arithmetic means were different from CNS and ***S. agalactiae*** (3.20) but both had equal log_{10}SCC arithmetic means ($P>0.05$).

The TBC geometric mean and median in bacteriologically negative quarters were 12,000 and 10,000cfu mL^{-1}, respectively. The values of descriptive statistics for TBC according to mastitis pathogens did not have the same order as SCC. The higher values of the geometric mean and median were for ***S. agalactiae***

Table 2 - Significance of differences between the means of mammary quarter somatic cell count (log_{10}SCC) and total bacterial count (log_{10}TBC) according to bacteriological examination results.

BER	Number of samples	----------Log_{10}SCC----------			----------Log_{10}TBC----------		
		Mean	SD	CI	Mean	SD	CI
NC	421	1.71a	0.88	1.63 - 1.80	1.09a	0.63	1.03 - 1.15
CNS	64	1.93b	0.80	1.73 - 2.13	1.22a	0.46	1.10 - 1.33
STA	69	2.77c	0.57	2.63 - 2.90	1.88b	0.72	1.71 - 2.06
STR	39	2.64c	0.72	2.40 - 2.87	2.03b	0.50	1.87 - 2.20
SAG	45	3.20d	0.60	3.03 - 3.39	2.52c	0.77	2.29 - 2.76

BER - bacteriological examination results; NG - no growth; CNS - coagulase negative staphylococci; STA - ***Staphylococcus aureus***; STR - other streptococci; SAG - ***Streptococcus agalactiae***; SD - standard deviation; CI - confidence interval (95%). Different letters between rows indicate statistic significance by two-tailed unpaired *t*-test ($P<0.05$).

Table 3 - Correlation and simple regression analysis for somatic cell count (log_{10}SCC) and total bacterial count (log_{10}TBC) according to bacteriological examination results.

BER	Non parametric analysis[1]	Parametric analysis	
		Pearson Correlation	Regression equation
NC + CNS	0.632**	0.626**	y = 0.338 + 0.440x ***
NC + STA	0.677**	0.659**	y = 0.263 + 0.503x ***
NC + STR	0.665**	0.654**	y = 0.295 + 0.486x ***
NC + SAG	0.674**	0.677**	y = 0.217 + 0.542x ***

BER - bacteriological examination results; NG - no growth; CNS - coagulase negative staphylococci; STA - ***Staphylococcus aureus***; STR - other streptococci; SAG - ***Streptococcus agalactiae***; [1] Spearman´s rank correlation; ** (P<0.01); *** (P<0.001).

(333,000 and 590,000cfu mL^{-1}). Other streptococci (108,000 and 100,00cfu mL^{-1}) and ***S. aureus*** (77,000 and 66,000cfu mL^{-1}) presented with higher TBC values. The TBC geometric mean (17,000cfu mL^{-1}) and median (15,000cfu mL^{-1}) for CNS were as for SCC, in other words, close to bacteriologically negative quarter values. Analysis of the arithmetic mean differences between the log_{10}TCB revealed a significance between bacteriological examination results (P<0.05). The arithmetic mean of the log_{10}TBC (Table 3) according to bacteriological examination results increased in a different order than the log_{10}SCC. The order was as follows: no growth, CNS, ***S. aureus***, other streptococci and ***S. agalactiae***. In this situation, no difference (P>0.05) was observed between the log_{10}TBC arithmetic mean for no growth (1.09) and CNS (1.22). ***S. aureus*** and other streptococci had no difference in the log_{10}TBC arithmetic mean (P>0.05). However, these were different (P<0.05) from the no growth, CNS and ***S. agalactiae*** arithmetic means. The geometric mean and median, which are relatively non-affected by extreme high values, better indicated the central number of the SCC and TBC found in mammary quarter milk samples than did the arithmetic mean. Large standard deviations were found for each mastitis pathogen in SCC and TBC, indicating that the data set had a great deal of variation. Because of the wide range of SCC and TBC results, logarithmic transformation was performed for comparison between means and the association between SCC and TBC. The corresponding Spearman´s rank correlations and Pearson correlations were in agreement (Table 2). Spearman and Pearson correlations used the logarithmic transformation of SCC and TBC. The correlation coefficients found between log_{10}SCC and log_{10}TBC for ***S. agalactiae*** (0.674, Spearman; 0.677, Pearson), ***S. aureus*** (0.677, Spearman; 0.659, Pearson), other streptococci (0.665, Spearman; 0.654, Pearson) and CNS (0.632, Spearman; 0.626, Pearson) were moderately high (P<0.01). The linear regression model significances were high (P<0.001) and showed different slopes for each mastitis pathogen (Table 3). In order, the higher slope was found for ***S. agalactiae*** (0.542), ***S. aureus*** (0.503), other streptococci (0.486) and CNS (0.440).

DISCUSSION

In mammary quarter milk samples that were not observed to have growth in the bacteriological examination, the geometric mean of the SCC was 52,000 cells mL^{-1}. The 95% confidence interval of individual values based on standard deviation varied between 205,000 and 357,000 cells mL^{-1}. A meta-analysis study on quarter milk SCC in dairy cows found 68,000 cells mL^{-1} to be the geometric mean in the absence of a diagnosed infection (DJABRI et al., 2002). The summary SCC values in bacteriologically negative quarters were between 68,000 and 187,000 cells mL^{-1}. The SCC geometric means of mammary quarters classified as not infected in this study and by DJABRI et al., (2002) were close and it could be considered equal in theoretical and practical approach. The SCC geometric mean found in the present study for ***S. agalactiae*** (1,572,000 cells mL^{-1}) was also close to the value found in the meta-analysis study (1,129,000 cells mL^{-1}). Among the causative pathogens of subclinical cases, such as ***S. aureus***, ***S. agalactiae***, CNS and other streptococci (HARMON, 1994), SCC values for ***S. agalactiae*** were higher than other mastitis pathogens in both studies. The highest SCC identified among mastitis pathogens found by DJABRI et al. (2002) was caused by ***Escherichia coli***, but this pathogen usually promotes clinical mastitis due to virulence factors and consequently pathophysiological processes (HARMON, 1994; HOGAN & SMITH, 2003). The SCC geometric mean for ***S. aureus*** (587,000 cells mL^{-1}) was twice as high as that found in the meta-analysis study (333,000 cells mL^{-1}). The inverse situation was observed for CNS; in the meta-analysis study, the SCC geometric mean was 155,000 cells mL^{-1}, and in present study it

was 85,000 cells mL^{-1}. However, the 95% confidence intervals for the SCC according to these mastitis pathogens had a high concordance of values with those reported by DJABRI et al. (2002). The SCC geometric mean for other streptococci could not be compared with the results found because the present study did not identify other streptococci than ***S. agalactiae***. The studies cited in the meta-analysis evaluated SCC from mammary quarters infected by ***Streptococcus uberis*** and ***Streptococcus dysgalactiae***. Although the present study did not identify ***S. uberis*** and ***S. dysgalactiae***, the SCC geometric mean value for other streptococci was 432,000 cells mL^{-1}. Data reported by DJABRI et al (2002) showed that SCC geometric means for ***S. uberis*** and ***S. dysgalactiae*** were 1,024,000 and 547,000 cells mL^{-1}, respectively. The SCC geometric mean for other streptococci was closer to that of ***S. dysgalactiae*** than ***S. uberis***, but the values contained in the confidence interval overlapped in both studies. Thus, it cannot be suggested, based on these results that most of the other streptococci were ***S. dysgalactiae*** or ***S. uberis***.

Considering the threshold of 70,000 cells mL^{-1} to sort infected mammary quarters, the results of this study are consistent with previous studies (SCHEPERS et al., 1997; DJABRI et al., 2002). The descriptive statistics showed wide variation in the results of SCC and TBC according to bacteriological examination results. This variation suggests that bacteriological examination may have provided false positive and false negative results due to the use of a single quarter milk sample to determine the infection status and mastitis pathogens, such as ***S. agalactiae*** and ***S. aureus***, in chronic infections that were shedding bacteria in a cyclic pattern (DINSMORE et al., 1991; HARMON, 1994; SEARS et al., 1990). Moreover, milk samples from non-infected mammary quarters obtained without washing or with improper washing of teats had higher TBC (7,000cfu mL^{-1}) than cows with teats dipped and dried with paper towels properly (1,500cfu mL^{-1}) (MCKINNON et al., 1988). Thus, bacteria, such as CNS, other streptococci and ***Corynebacterium*** spp., may have contributed to higher TBC values in the milk samples with no growth of mastitis pathogens due to the contamination of these pathogens from a part of the resident bacterial flora on the teat skin (HARMON, 1994).

Results on the shedding of bacteria by the mammary quarters according to bacteriological examination results showed similarity with the results of SCC. The main difference in the results of TBC in relation to SCC was for ***S. aureus*** and other streptococci. In this case, the shedding of bacteria from infected mammary quarters by other streptococci was higher when compared with those infected with ***S. aureus***. The other pathogens isolated (***S. agalactiae*** and CNS) presented the higher and lower shedding of bacteria and SCC, respectively. The geometric mean found for milk samples with the isolation of mastitis pathogens ranged from 17,000 to 333,000cfu mL^{-1}. The variation in the TBC results are in agreement with KEEFE (1997) and BRAMLEY et al. (1990), who indicated that the shedding of bacteria by infected cows ranges from 10^2 to 10^7 per mL. GONZALEZ et al. (1986) found a moderately high correlation obtained for ***S. agalactiae***, indicating that values greater than 4,000cfu mL^{-1} in bulk milk tank indicates that at least 7% of cows were shedding this bacteria. This correlation was fewer compared to ***S. aureus***, other streptococci, CNS and coliform bacteria. However, ***S. uberis*** was identified as a mastitis pathogen causing TBC peaks in bulk tank milk, ranging from 14,000 to 600,000cfu mL^{-1} (HAYES et al., 2001; ZADOKS et al., 2004). Thus, the impact of mastitis pathogens on bulk tank TBC depends on the percentage of infected mammary quarters and type of bacterial mastitis pathogen involved. The stage of infection can also contribute, with an increased shedding of bacteria from the mammary gland (SEARS et al., 1990; KEEFE, 1997) causing an increase in the bulk tank TBC (RYSANEK et al., 2005).

Correlation results showed that when SCC were higher, the number of mastitis pathogens per milliliter of milk sample was also expected to be high. Thus, SCC was a good indicator of shedding bacteria from mammary glands, but these correlations were not sufficient to assess differences between mastitis pathogen with the same increasing SCC. However, linear regression slopes predicted that the same increasing SCC was associated with different numbers of bacteria being shed from the mammary gland according to mastitis pathogen. These results showed that ***S. agalactiae*** and other streptococci can cause TBC peaks in bulk tank milk (HAYES et al., 2001; ZADOKS et al., 2004). As mentioned previously, the magnitude of the peak of bulk tank milk TBC will depend on the percentage of infected mammary quarters, the strain of bacteria and stage of infection.

CONCLUSION

In conclusion, the greater the intensity of the inflammatory process, the greater was the shedding of bacterial mastitis pathogens from the mammary gland. However, the magnitude of shedding of mastitis pathogens from the mammary gland was different according to mastitis pathogens. The combination of SCC and TBC from mammary quarters may be useful as screnning test for streptococci mastitis. More studies are necessary to better understand the variation in the shedding of mastitis pathogens according to the age, parity, lactation stage and infection stage of the cow.

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