

## A time series analysis of bulk tank somatic cell counts of dairy herds located in Brazil and the United States

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**ABSTRACT:** Bulk tank somatic cell counts (BTSCC) is widely used to monitor the mammary gland health at the herd and regional level. The BTSCC time series from specific regions or countries can be used to compare the mammary gland health and estimate the trend of subclinical mastitis at the regional level. Three time series of BTSCC from dairy herds located in the USA and the Southeastern Brazil were evaluated from 1995 to 2014. Descriptive statistics and a linear regression model were used to evaluate the data of the BTSCC time series. The mean of annual geometric mean of BTSCC (AGM) and the percentage of dairy herds with a BTSCC greater than 400,000 cells mL<sup>-1</sup> (>400) were significantly different ( $P < 0.05$ ) according to the countries and the times series. Linear regression model used for the USA time series was statistically significant for AGM and the >400 ( $P < 0.05$ ). The first and second USA time series presented an increasing and decreasing trend for AGM and the >400, respectively. The linear regression model for the Brazil time series was not significant ( $P > 0.05$ ) for both dependent variables (AGM and >400). The Brazil time series showed no increasing or decreasing trend for the AGM and >400. Consequently, approximately 40 to 50% of the dairy herds from southeastern Brazil will not achieve the regulatory limits for BTSCC over the next years.

**Key words:** bulk tank somatic cell counts, time series, bovine mastitis, Brazil, United States.

### Uma análise de séries temporais de contagem de células somáticas de tanque de rebanhos leiteiros localizados no Brasil e nos Estados Unidos

**RESUMO:** Contagens de células somáticas de tanque (BTSCC) são amplamente utilizadas para monitorar a saúde da glândula mamária em níveis regional e de rebanho. Séries temporais de BTSCC, de regiões ou países específicos, podem ser usadas para comparar a saúde da glândula mamária e estimar a tendência de mastite subclínica em nível regional. Três séries temporais de BTSCC de rebanhos leiteiros, localizados nos EUA e na região sudeste do Brasil, foram avaliadas de 1995 a 2014. A estatística descritiva e modelos de regressão linear foram usados para avaliar as informações das séries temporais de BTSCC. A média anual geométrica de BTSCC (AGM) e a porcentagem de rebanhos com BTSCC, maior que 400.000 células mL<sup>-1</sup> (>400), foram significativamente diferentes ( $P < 0,05$ ) de acordo com os países e as séries temporais analisadas. O modelo de regressão linear usado para as séries temporais dos EUA foram estatisticamente significantes para AGM e >400 ( $P < 0,05$ ). A primeira e a segunda séries temporais dos EUA apresentaram uma tendência de aumento e diminuição, tanto para AGM quanto para >400, respectivamente. O modelo de regressão linear para as séries temporais do Brasil não foi significante ( $P > 0,05$ ) para ambas as variáveis dependentes (AGM e >400). As séries temporais do Brasil não apresentaram tendência de aumento ou diminuição para AGM e >400. Consequentemente, aproximadamente, 40 a 50% dos rebanhos leiteiros do sudeste do Brasil não alcançarão os limites regulatórios para BTSCC ao longo dos próximos anos.

**Palavras-chave:** contagem de células somáticas de tanque, séries temporais, mastite bovina, Brasil, Estados Unidos.

## INTRODUCTION

Mastitis is an endemic disease in dairy herds worldwide and causes, among other problems, the greatest economic loss related to production diseases (BENNET et al., 1999; HALASA et al., 2007). Economic decisions related to the control of mastitis are based on the cost of clinical and subclinical cases and the cost of procedures (HALASA et al., 2007). Decisions related to the control of mastitis can occur at the cow, farm, regional or country level (HALASA et al., 2007). For instance, decisions at the regional or country level are those that are mainly related to data collection and include data collection (database)

from a given region and its population to investigate the benefits of mastitis control programs (BEEK et al., 1992; KEEFE et al., 1997; HALL et al., 2004). Somatic cell counts (SCC) are widely recognized as a subclinical mastitis indicator and are used in the evaluation and monitoring of the health of the mammary gland in dairy herds in several countries (SCHUKKEN et al., 2003). In an effort to improve product quality and, indirectly, farm productivity, regulatory limits on SCC were established by many of the major dairy producing countries. In Europe, the Council Directive 92/46 of the Council of the European Communities in April 1992 stated that milk with SCC over 400,000 cells mL<sup>-1</sup> may not be

used for fluid milk and, starting in 1998, not even for human consumption. In North America, limits are placed at 750,000 cells mL<sup>-1</sup> (USA) and 500,000 cells mL<sup>-1</sup> (Canada) (SARGEANT et al., 1998). Since 2005 in Brazil, the bulk tank somatic cell counts (BTSCC) are regarded in its legislation, and the limits are gradually being reduced. In July of 2016, a limit of 400,000 cells mL<sup>-1</sup> will be established for dairy herds located in the southeastern region (BRAZIL, 2011). Monitoring SCC at the herd and regional level requires longitudinal data (SCHUKKEN et al., 2003). A time series analysis of BTSCC of dairy herds from a specific region or country can provide information about a situation and a trend of subclinical mastitis at the regional or country level and is useful to make decisions on the mastitis control program. BERRY et al. (2006) evaluated the BTSCC for a decade in dairy herds from Ireland and observed a decrease in BTSCC from 1994 to 2000. However, there was a slow increase in SCC from 2000 to 2004. Countries that have legislation relating to raw milk, which establishes a limit for BTSCC, can use a time series to assess the situation and trends of herds that do not achieve these limits. SCHUKKEN et al. (1993) evaluated the dynamics and regulations of BTSCC from 1985 until 1991 in dairy herds from Canada using a time series cross-sectional analysis. In this study, the authors reported that the regulatory program had a substantial impact on the BTSCC levels. Comparing the time series of BTSCC of dairy herds from different regions or countries can be useful to identify similarities and/or differences related to the health of the mammary gland and help make decisions at the regional or country level for the mastitis control program. The objective of the present study was to evaluate the differences between a time series of BTSCC from dairy herds located in the southeastern region of Brazil and the United States from 1995 until 2014.

## MATERIALS AND METHODS

### *Herds and data from Brazil and the United States*

Data regarding BTSCC from 2006 to 2014 of dairy herds located in the Southeastern region of Brazil were obtained from the Milk Quality Laboratory of Embrapa Dairy Cattle. The dairy herds used in the statistical analysis had at least six milk samples from the bulk tank per year. Volumes of 40mL of milk were obtained from each herd after the bulk milk tank had been homogenized. Samples were preserved with Bronopol (Bronopol, D&F Control Systems, Dublin, USA) and maintained under refrigeration

until processing at the laboratory. The SCC were determined by flow cytometry using a Bentley Combi 2300 (Bentley Instruments, Chaska, USA) (IDF, 1995). Data regarding BTSCC from 1995 to 2013 of dairy herds located in the United States were obtained from the Council on Dairy Cattle Breeding Research Report (NORMAN & WALTON, 2014). The time series of BTSCC from the United States dairy herds used in this study was related to the annual geometric mean of BTSCC and the percentage of dairy herds with an annual geometric mean greater than 400,000 cells mL<sup>-1</sup>. Number of herds of each country and year, annual geometric mean of BTSCC (AGM) and percentage of dairy herds with a geometric mean of SCC greater than 400,000 cells mL<sup>-1</sup> (%>400) are presented in table 1.

### *Statistical analyses*

Data from the United States dairy herds were divided into two time series. The first and second time series were from 1995 to 2003 and from 2004 to 2014, respectively. Descriptive statistics were performed using the number of dairy herds, AGM and %>400 according to the countries and the time series (Table 2). The normality of the data of the AGM and %>400 for each time series distribution was evaluated by the Shapiro-Wilk test. Significance of the arithmetic mean differences between AGM and %>400 according to time series was determined by an independent samples *t*-test. Levene's test was used to evaluate the variance between time series. Linear regression analyses were conducted using the AGM and %>400 as the dependent variables (*y*) and the year as the independent variable (*x*). Thus, three linear regression analyses were performed to compare the slope among the time series according to the dependent variables. The linear regression and angular coefficients were evaluated for statistical significance. Statistical analyses were performed using SPSS version 8.0 (SPSS, 1998).

## RESULTS AND DISCUSSION

Three time series evaluated in this study presented a normal distribution for the number of herds according to the results of the Shapiro-Wilk test ( $P>0.05$ ). Levene's test showed equal variance for both of the USA time series when compared with the Brazil time series for %>400 but different variances between the USA and the Brazil time series for AGM.

The three time series evaluated in this study presented a normal distribution for AGM and %>400 according to the results of the Shapiro-Wilk

Table 1 - Number of dairy herds, annual geometric mean of bulk tank somatic cell counts ( $\times 1,000$  cells  $\text{mL}^{-1}$ ) and percentage of dairy herds with bulk tank somatic cell counts greater than 400,000 cells  $\text{mL}^{-1}$  according to the country and year.

Year	-----United States-----			-----Brazil-----		
	N	AGM	%>400	N	AGM	%>400
1995	265,844	304	27.2	-	-	-
1996	255,039	308	27.8	-	-	-
1997	287,789	314	28.8	-	-	-
1998	283,695	318	30.3	-	-	-
1999	273,364	311	29.8	-	-	-
2000	260,139	316	29.5	-	-	-
2001	244,940	322	31.1	-	-	-
2002	267,809	320	30.0	-	-	-
2003	251,182	319	30.4	-	-	-
2004	240,938	295	26.4	-	-	-
2005	234,585	296	25.8	-	-	-
2006	236,191	288	25.2	12,895	512	53.4
2007	227,626	276	24.0	15,285	521	57.6
2008	222,245	262	22.4	15,976	468	49.6
2009	204,195	233	18.9	15,771	564	59.8
2010	198,218	228	18.0	16,019	473	48.0
2011	191,375	217	15.7	15,715	571	61.7
2012	184,927	200	12.0	16,390	528	55.1
2013	177,944	199	11.6	14,510	498	51.3
2014	-	-	-	14,104	536	55.5

N - Number of dairy herds; AGM - Annual geometric mean; %>400 - Percentage of dairy herds with bulk tank somatic cell counts greater than 400,000 cells  $\text{mL}^{-1}$ .

test ( $P > 0.05$ ). Levene's test showed equal variance for the USA time series from 2004 to 2013 and the Brazil time series. Different variances were observed when compared to the USA time series from 1995 to 2003 and the Brazil time series for both variables. In this situation, the significance of the difference between the means of the USA time series when compared with mean of the Brazil time series did not show equal variances.

The annual geometric mean of BTSCC according to the countries and the time series were different, as shown by an independent samples *t*-test ( $P < 0.05$ ) (Table 2). Results showed a significant reduction of AGM from the USA dairy herds from the first to the second time series. The means of %>400 according to the countries and time series were also different, as shown by the independent samples *t*-test

Table 2 - Descriptive statistics and linear regression of the annual geometric mean of the bulk tank somatic cell counts ( $\times 1,000$  cells  $\text{mL}^{-1}$ ) and the percentage of dairy herds with bulk tank somatic cell counts greater than 400,000 cells  $\text{mL}^{-1}$  according to the countries and time series.

Variable	Country/Time series	N	Mean*	SD	Linear regression	P1	R <sup>2</sup>
AGM	USA/1995-2003	9	314 <sup>b</sup>	6	$y = 305.5 + 1.83x$	<0.01	0.705
	USA/2004-2013	10	249 <sup>a</sup>	38	$y = 318.2 - 12.51x$	<0.001	0.966
	Brazil/2006-2014	9	518 <sup>c</sup>	35	$y = 506.1 + 2.56x$	0.609	0.039
%>400	USA/1995-2003	9	29.4 <sup>b</sup>	1.3	$y = 27.5 + 0.39x$	<0.01	0.691
	USA/2004-2013	10	20.0 <sup>a</sup>	5.6	$y = 29.9 - 1.81x$	<0.001	0.960
	Brazil/2006-2014	9	54.7 <sup>c</sup>	4.6	$y = 54.4 + 0.04x$	0.951	0.001

AGM - Annual geometric mean of bulk tank somatic cell counts; %>400 - Percentage of dairy herds with bulk tank somatic cell counts greater than 400,000 cells  $\text{mL}^{-1}$ ; N - Number of years in the time series; SD - Standard deviation; \*Different letters between the lines indicate statistical differences ( $P < 0.05$ ); P1 - Model significance level; R<sup>2</sup> - Determination coefficient (model adjustment).

( $P < 0.05$ ) (Table 2). This result suggests that the situation regarding subclinical mastitis is different between both of the USA time series and between the Brazil and USA time series. An improvement of the mammary gland health in the USA during the entire time period was observed with a mean reduction of approximately 20% of AGM and 10% of dairy herds with a geometric mean of BTSCC greater than 400,000 cells  $\text{mL}^{-1}$  from the first to second time series. In all of the periods related to the first USA time series, an increase of 15,000 cells  $\text{mL}^{-1}$  and approximately 3% for AGM and %>400 was observed. In contrast, the second USA time series showed a decrease of 96,000 cells  $\text{mL}^{-1}$  and approximately 15% for AGM and %>400. However, a decrease of 4% was observed from the last year of the first USA time series (2003) to the first year of the second USA time series (2004).

The linear regression models of AGM were statistically significant ( $P < 0.01$ ) for the first and second USA time series with an adjustment of 70.5 and 96.6%, respectively. Linear regression models for %>400 were also statistically significant ( $P < 0.01$ ) for the first and second USA time series with an adjustment of 69.1 and 96.0%, respectively (Table 2). The first and second USA time series presented an increasing and decreasing trend of AGM, respectively. The same increasing and decreasing trends were observed for %>400 for USA dairy herds according to the time series. Linear regression model for the first USA time series estimated an increase of approximately 2,000 cells  $\text{mL}^{-1}$  for AGM and 0.4% of %>400 per year (Table 2). However, the second USA time series showed an inverse relationship with a decrease of approximately 12,500 cells  $\text{mL}^{-1}$  and 1.8% of AGM and %>400 per year (Table 2). Although the first and second USA time series showed an increase and decrease of the annual geometric mean of BTSCC and the percentage of dairy herds with BTSCC greater than 400,000 cells  $\text{mL}^{-1}$ , respectively, when the data of the USA time series were analyzed for the entire time period, a significant decrease of approximately 6,800 cells  $\text{mL}^{-1}$  and 1.0% for the dairy herds with a BTSCC greater than 400,000 cells  $\text{mL}^{-1}$  was observed per year (NORMAN & WALTON, 2014).

In the Brazil time series, the AGM and %>400 means were 518,000 cells  $\text{mL}^{-1}$  and 54.7%, respectively. Brazil AGM was 1.65 and 2.10 times greater than the first and second USA time series, respectively. The mean of the %>400 in the dairy herds located in the Southeastern region of Brazil was 25.3 and 34.7% greater than the first and second USA time series, respectively (Table 2). Linear regression model for the Brazil time series was not statistically significant

for either of the dependent variables ( $P > 0.05$ ). The statistical model used to evaluate the time series could be one more complex with better adjustment. However, the linear regression model used to Brazil time series was the same that used in USA time series (NORMAN & WALTON, 2014) with the objective to compare time series between countries.

*Staphylococcus aureus* and *Streptococcus agalactiae* are contagious mastitis pathogens for which the major reservoir is the infected udder, and the infections tend to be chronic and subclinical (HARMON, 1994). Since the introduction of a standard mastitis prevention program (NEAVE et al., 1969), there has been much progress in decreasing the prevalence of subclinical mastitis, which is mainly caused by *S. aureus* and *S. agalactiae*, and a decrease in the mean BTSCC in national milk production has been achieved (SCHUKKEN et al., 2003). Although the first USA time series from 1995 to 2003 showed a reduction in the number of herds over time (almost 15,000 dairy herds), there was a slight increase in the AGM and %>400. In this period at the country level, no improvement was observed in the subclinical mastitis situation. This situation suggested that the control and prevention procedures of subclinical mastitis were not homogeneously and efficiently adopted in the country, which could be observed in the BTSCC time series. In contrast, the second USA time series showed a decrease in the number of dairy herds, AGM and %>400. In this time series, a significant improvement in the subclinical mastitis situation was observed from 2004 to 2013.

In 2007, the National Animal Health Monitoring System (NAHMS) performed a national study to estimate the prevalence of mastitis contagious pathogens (USDA, 2008). Of the three contagious mastitis pathogens, *S. aureus* had the highest herd level prevalence at 43.0% of the dairy herds; whereas, *S. agalactiae* and *Mycoplasma* spp. were reported in 2.6 and 3.2% of dairy herds, respectively. In the same year, 2007, the AGM was 276,000 cells  $\text{mL}^{-1}$ , and 24% of the USA dairy herds presented an annual geometric mean of BTSCC greater than 400,000 cells  $\text{mL}^{-1}$ . Therefore, the AGM and %>400 for USA dairy herds for 2007 were associated with prevalence of 43.0, 2.6 and 3.2% for *S. aureus*, *S. agalactiae* and *Mycoplasma* spp., respectively. The second USA time series presented a decrease from 26.4 to 11.6% (nearly 15%) for dairy herds that presented a BTSCC greater than 400,000 cells  $\text{mL}^{-1}$ , suggesting that the prevalence of subclinical mastitis, and consequently the prevalence between herds for contagious mastitis pathogens, also decreased. Dividing the data of

the USA dairy herds into two time series allowed identification of a critical period where there was an increase of AGM and %>400, and after this period, a decrease of both variables was observed, indicating an improvement of mammary gland health.

The main approach for the control and prevention of *S. agalactiae* should be directed toward eradication of this pathogen through treatment of infected cows. In contrast, the approach for the control measure of *S. aureus* should be based on the culling of cows with chronic infection due to its difficult elimination (OLIVEIRA et al., 2013). The decrease of approximately 63,000 USA dairy herds and the consequent elimination of chronically infected dairy cows from these herds can be one of the causes responsible for the decrease of AGM and %>400. However, this association with the elimination of chronically infected dairy cows suggested that the main control measures showed homogeneous and efficient improvement at the country level (HALASA et al., 2007), mainly from 2004, even when a significant decrease of AGM and %>400 for the next ten years was observed. The percentage of USA dairy herds that exceeded the legal limit would also have been higher than the percentage of herds that had milk rejected from the market because market exclusion only occurs after repeated violations (NORMAN & WALTON, 2014). The Brazil time series of AGM and %>400 showed that the control of subclinical mastitis is one of the challenges of the Brazilian dairy industry. This critical situation is mainly due to a high percentage of dairy herds with a BTSCC greater than 400,000 cells mL<sup>-1</sup>, which is the regulatory limit established by Brazilian legislation for these dairy herds after July of 2016 (BRAZIL, 2011). When comparing the BTSCC parameters from the Brazil dairy herds with both of the USA time series, results suggested that the prevalence between dairy herds for *S. aureus* and *S. agalactiae* were greater than 43.0 and 2.6%, respectively. A high %>400 was observed, ranging from 48.0 to 61.2%, which suggested that the specific procedures according to contagious mastitis pathogens are not widespread and were not adopted efficiently in dairy herds in the Southeastern region of Brazil.

The situation related to AGM and %>400 in dairy herds located at the Southeastern region of Brazil will not increase or decrease suddenly according to the linear regression models. Regarding the confidence interval of 95% for the mean, the AGM and %>400 will range from 491,000 and 546,000 cells mL<sup>-1</sup> and from 51.1 to 58.2%, respectively, for the next couple of years if mastitis control procedures at the herd and regional level are not adopted, which is mainly related

to contagious pathogens. Even if a control mastitis program is adopted at the regional level, if there is not a heavy culling of cows with chronic infection, the AGM and %>400 will not decrease suddenly. Considering the second USA time series of BTSCC, a decrease of 20% of herds with a BTSCC greater than 400,000 cells mL<sup>-1</sup> is possible within 10 years. In southeastern Brazil, if the design and adoption of a mastitis control program at the herd and regional level occurs in a short time, the decrease of BTSCC will occur over a medium or long time. Therefore, in regards to the regulatory limits established in Brazil, a trend of maintaining the percentage range from 50 to 60% of dairy herds would occur without achieving the limits of the BTSCC of 400,000 cells mL<sup>-1</sup> for the next years. Finally, the location and number of dairy herds used in the USA time series provided adequate information to make an inference for the USA dairy herds. The location and number of dairy herds used in the Brazil time series provided adequate information to make an inference for the dairy herds located in the Southeastern region of Brazil, which is an important region for milk production in Brazil.

## CONCLUSION

Subclinical mastitis in dairy herds from the USA and the southeastern region of Brazil presented differences according to a time series. Dairy herds from the Southeastern region of Brazil did not present a trend of improvement related to a decrease of BTSCC, and approximately 50% of the dairy herds will not achieve the regulatory limits. Monitoring BTSCC at the regional or country level over time provides an opportunity to evaluate progress in the dairy industry and study relationships between the parameters of dairy herds and estimate the efficacy of mastitis control programs.

## ACKNOWLEDGEMENTS

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 014/2010 - Process 477493/2010-6) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG - APQ CVZ 01305/2010).

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