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Decision-Making in Mastitis Prevention and Control at Regional, Herd and Individual Levels Based on Epidemiological and Economic Studies

Mendonça JFM[1], Brito MAVP[2], Lange CC[2], Silva MR[2], Mendonça LC[2], Ribeiro JB[2], Siqueira LGB[2], Sias GRFV[4], Soares LAP[5] and Souza GN[*2,3]

[1]Veterinary Hygiene and Technology Processing of Animal Products, Fluminense Federal University, Niterói, RJ, Brazil
[2]Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil
[3]Department of Veterinary Collective Health and Public Health, School of Veterinary Medicine, Federal Fluminense University, Niterói, RJ, Brazil
[4]Juiz de Fora Federal University, Veterinary Medicine Department, Juiz de Fora, MG, Brazil
[5]Santa Catarina State University, Veterinay Medicine School, Lages, SC, Brazil

[*]**Corresponding author:** Souza GN, Santa Catarina State University, Veterinay Medicine School, Lages, SC, 88520-000, Brazil, Tel: +553233117537, E-mail: guilherme.souza@embrapa.br

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Abstract

The objective of this review is to describe results of epidemiological and economic studies carried out in Brazil that can assist in the decision making process at the region, herd and individual levels for the prevention and control of bovine mastitis. At regional and herd-levels, we present data from a time series analysis comparing somatic cell counts in herds located in Brazil and in the United States (US), an estimation of the prevalence of contagious mastitis pathogens in a specific population of herds located at the state of Minas Gerais, and finally we report the identification of risk factors for new and chronic intramammary infections within herds located at the state of Santa Catarina. The outcomes of epidemiological studies that support the decision making process at herd- and individual-level are related to the decrease in prevalence of contagious mastitis pathogens in the herd as well as the estimate of the economic impact of mastitis occurrence.

Keywords: *Streptococcus agalactiae; Staphylococcus aureus*; Risk Factors; Prevalence; Time Series

Introduction

The knowledge generated from specific epidemiological studies carried out at different countries, including Brazil, on somatic cell count (SCC) from cows and bulk tank samples, infection patterns and prevalence of contagious mastitis pathogens among distinct dairy herds and among animals within herds, identification of risk factors for mastitis, and evaluation of the economic impact of mastitis provided crucial information about the occurrence of the disease. The results obtained in previous studies may be used to improve mastitis prevention and control programs [1-16].

Mastitis is an endemic disease in dairy herds worldwide and causes, among other problems, the greatest economic loss related to production diseases [14,17]. Economic decisions related to the control of mastitis are based on the cost of clinical and subclinical cases and the cost of the management procedures [14]. Decisions related to the control of mastitis can occur at the regional-, country-, farm-, and cow-level [14]. For instance, decisions at the regional or country levels are those mainly related to investigations of databases and include data collection from a given region and its population to determine the benefits of mastitis control programs [6,18-20]. Decisions at the farm level are those related to disease prevention and control procedures [21]. These control procedures allow the reduction in the incidence of clinical and subclinical cases and the improvement of milk quality [22]. Finally, decisions at the cow level are those related to an individual animal such as treatment of clinical or subclinical cases and even the decision of culling affected cows [9]. Treatment of an individual cow, however, may also be considered as a farm-level decision, because this measure also prevents new cases of mastitis within the herd.

Actually, in Brazil there is extensive information in the literature on mastitis that may support the decision making process for mastitis prevention and control at the regional-, herd- and individual-level. Therefore, the present work aims to discuss results of epidemiological and economic studies carried out in Brazil on mastitis prevention and control that can aid decision making processes to deal with this disease in dairy cattle herds.

Regional level

A time series comparative analysis of bulk tank SCC from dairy herds located in Brazil and the United States of America (US) [6].

In an effort to improve product quality and, indirectly, farm productivity, regulatory limits on SCC have been 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 > 400,000 cells/mL may not be used for fluid milk sales and, starting in 1998, not even for human consumption. In North America, limits were defined at 750,000 cells/mL (US) and 500,000 cells/mL (Canada) [23]. In Brazil, bulk tank SCC (BTSCC) is regulated by the animal products legislation since 2005 and the limits are gradually being reduced to a maximum limit of 400,000 cells/mL to be achieved by the year 2019 [24]. Monitoring SCC at the herd and regional level requires analysis of longitudinal data [2]. Thus, a time series analysis of BTSCC in dairy herds from an specific region or country may provide key information about a situation and establish a trend of subclinical mastitis at the regional or country level, which is a useful tool to make decisions about the mastitis control program regionnally. The study therefore aimed to evaluate differences between BTSCC data collected sequentially from dairy herds located in the southeastern region of Brazil or atthe US from 1995 to 2014.

BTSCC data from 2006 to 2014 in dairy herds located in southeastern Brazil were analyzed and recorded by the Embrapa Dairy Cattle Milk Quality Laboratory. Herds included in the statistical analysis had at least six bulk tank milk samples per year. Data regarding BTSCC from 1995 to 2013 from US dairy herds were obtained from the Council on Dairy Cattle Breeding Research Report [25]. The BTSCC time series from US dairy herds used in this study were selected by the annual BTSCC geometric mean (AGM) and the percentage of dairy herds with an annual geometric mean >400,000 cells/mL (%>400). The number of herds per country and year, AGM and % > 400 are shown in Table 1.

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/mL

Table 1: Number of dairy herds, annual geometric mean (AGM) of bulk tank somatic cell count (SCC; x 1,000 cells/mL) and percentage of dairy herds with bulk tank SCC ≥400,000 cells/mL by the country and year

AGM differed between countries and was affected by time (Table 2). Results demonstrated a significant decrease in AGM at US herds from the first to the second time series ($P<0.05$). The % > 400 did also differ between countries and time series (Table 2). These results suggest that the situation regarding subclinical mastitis is different between the two US time series and between time series from Brazil and the US. Mammary gland health improved over time in the US, where an average reduction of approximately 20% was observed in AGM data and a 10% decrease in dairy herds with a BTSCC geometric mean >400,000 cells/mL was detected from the first to the second time series. Within all periods in the first US time series, we observed an increase of 15,000 cells/mL and approximately 3% for AGM and % > 400. In contrast, the second US time series indicated a decrease of 96,000 cells/mL and approximately 15% for AGM and % > 400.

Variable	Country/Time series	N	Mean*	SD	Linear regression	P-value[1]	R^2
AGM	US/1995-2003	9	314[b]	6	y = 305.5 + 1.83x	<0.01	0.705
	US/2004-2013	10	249[a]	38	y = 318.2 – 12.51x	<0.001	0.966
	Brazil/2006-2014	9	518[c]	35	y = 506.1 + 2.56x	0.609	0.039
% > 400	US/1995-2003	9	29.4[b]	1.3	y = 27.5 + 0.39x	<0.01	0.691
	US/2004-2013	10	20.0[a]	5.6	y = 29.9 – 1.81x	<0.001	0.960
	Brazil/2006-2014	9	54.7[c]	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/mL; 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^2 – Determination coefficient (model adjustment)

Table 2: Descriptive statistics and linear regression of the annual geometric mean of bulk tank somatic cell count (SCC; ×1,000 cells/mL) and the percentage of dairy herds with bulk tank somatic cell counts ≥400,000 cells/mL according to countries and time series

The AGM linear regression models were statistically significant ($p < 0.01$) for the first and second US time series with an adjustment of 70.5 and 96.6%, respectively. The linear regression models for % > 400 were also statistically significant ($p < 0.01$) for the first and second US time series with an adjustment of 69.1 and 96.0%, respectively (Table 2). The first US time series had an increasing trend for AGM, whereas the trend was decreasing in the second time series. Similar increasing and decreasing trends were observed for % > 400 in US dairy herds according to the time series. A linear regression model for the first US time series estimated an increase of approximately 2,000 cells/mL for AGM and 0.4% of % > 400 per year (Table 2). In contrast, the second US time series revealed an inverse relationship with a decrease of approximately 12,500 cells/mL and 1.8% of AGM and % > 400 per year (Table 2). Although the first and second US time series indicated an increase and decrease of the AGM of BTSCC and the % > 400, respectively, when data from both US time series were combined, analysis revealed a significant decrease of approximately 6,800 cells/mL and 1.0% for the dairy herds with a BTSCC greater than 400,000 cells/mL per year [25].

In the Brazil time series, AGM and % > 400 means were 518,000 cells/mL and 54.7%, respectively. The Brazil AGM was 1.65 and 2.10 times greater than the first and second US time series, respectively. The mean of the % > 400 in dairy herds located in southeastern Brazil was 25.3 and 34.7% greater than the first and second US time series, respectively (Table 2). The linear regression model for the Brazil time series was not statistically significant for neither of the dependent variables ($p > 0.05$).

Although the first US time series from 1995 to 2003 indicated a reduction in the number of existing herds over time (decrease of ~15,000 dairy herds), there was a slight increase in AGM and % > 400. In this period at the country level, no improvement was observed in the subclinical mastitis situation. This situation suggests that procedures for prevention and control of subclinical mastitis were not adopted homogeneously and efficiently across the country, which could be observed in the BTSCC time series. In contrast, the second US time series revealed 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 contagious mastitis pathogens [26]. Of the three major pathogens, *S. aureus* had the highest herd-level prevalence in 43.0% of the dairy herds, whereas *S. agalactiae* and *Mycoplasma* spp. were found in 2.6 and 3.2% of the herds, respectively. In that same year, AGM was 276,000 cells/mL and %>400 of the US dairy herds was 24%.The second US time series indicated a decrease from 26.4 to 11.6% (nearly a 15% reduction) for dairy herds with BTSCC greater than 400,000 cells/mL, suggesting a decrease in the prevalence of subclinical mastitis and, consequently, the prevalence of contagious mastitis pathogens among herds. Dividing the data of US dairy herds into two time series allowed the identification of a critical period where there was an increase in AGM and % > 400 and, after this period, a decrease in both variables, indicating an overall improvement of mammary gland health.

The main approach for the prevention and control of *S. agalactiae* should be directed towards the eradication of this pathogen through treatment of infected cows. In contrast, the control measure for *S. aureus* should be based on culling cows with chronic infection due to its difficult elimination [8]. The decrease of approximately 63,000 US dairy herds and the subsequent elimination of chronically infected dairy cows from these herds can perhaps be one of the causes responsible for the decrease in AGM and % > 400. Nevertheless, this association with elimination of chronically infected dairy cows suggests that the main control measures led to homogeneous and efficient improvement at the country level, mainly after 2004, when a significant decrease in AGM and % > 400 was observed in the following ten years [14]. The percentage of US dairy herds exceeding legal limits might also have been higher than the percentage of herds having milk rejected from the market, because market exclusion only occurs after repeated violations [25].

The Brazil time series of AGM and % > 400 demonstrated that control of subclinical mastitis is one of the challenges for 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, which is the regulatory limit established in Brazilian bylaws for these dairy herds after July of 2016 [24]. When comparing BTSCC parameters from Brazilian dairy herds with both US time series, results suggest that the prevalence for *S. aureus* and *S. agalactiae* were greater than 43.0 and 2.6%, respectively, as reported by USDA [26]. The % > 400 ranged from 48.0

to 61.2%, which suggests that specific procedures recommended to prevent contagious mastitis pathogens are not widespread and neither adopted efficiently in dairy herds in the southeastern region of Brazil.

The situation of AGM and % > 400 in dairy herds located in southeastern Brazil is not likely to increase or decrease suddenly, based on linear regression models. In regard to 95% confidence intervals, AGM ranges from 491,000 to 546,000 cells/mL and % > 400 between 51.1 and 58.2%, for the next years if mastitis control procedures at the herd and regional level were not adopted, mainly related to contagious pathogens. Even if a mastitis control program was adopted in the region, but a substantial culling of cows with chronic infection does not occurs, AGM and % > 400 are not going to decrease rapidly.

Considering BTSCC data in the second US time series, a 20% decrease in the number of herds with BTSCC greater than 400,000 cells/mL is possible within a 10-year period. In southeastern Brazil, if a mastitis control program was designed and adoptedat the herd and regional levels in the short-term, the decrease in BTSCC may occur in the medium- or long-term. Therefore, taking into account the regulatory limits established in Brazil, a trend of maintaining 50 to 60% of dairy herds over the BTSCC limit of of 400,000 cells/mL remains for the next years. Finally, the location and number of dairy herds used in the US time series provide reliable information to make inferences about US dairy herds, whereas location and number of dairy herds used in the Brazil time series provide information about dairy herds located in the southeastern region of Brazil, which is the 2nd largest milk production region in the country.

Subclinical mastitis in dairy herds from the US and southeastern Brazil had clear differences among time series. Brazilian dairy herds did not present a trend of improvemen in BTSCC and approximately 50% of the herds do not meet regulatory limits. Nonetheless, monitoring BTSCC at the regional or country level over time provides an opportunity to evaluate the dairy industry progress and to study interrelationships among dairy herds indicators and eventually estimate the efficacy of mastitis control programs.

Regional and herd level

Estimating the prevalence of *Sthaphylococcus aureus* and *Streptococcus agalactiae* in dairy herds registered by the Minas Gerais Holstein Breeders Association, Brazil, 2011/2012 [8]

Bovine mastitis is a disease that requires constant monitoring mainly due to the contagious pattern of *S. aureus* and *S. agalactiae*. The identification of these agents in dairy herds in the state of Minas Gerais, southeastern Brazil, as well as the variation in SCC according to contagious mastitis pathogens have been previously reported [7]. The knowledge about the prevalence of contagious mastitis pathogens permits the quantification the disease among herds and may be used for decision making processes at the region and herd-level. Thus, this study investigated the prevalence of *S. aureus* and *S. agalactiae* amongst herds registered at the Minas Gerais Holstein Breeders Association (AMGHDF).

The studied population was composed of 112 dairy herds with nearly 6.000 lactating cows, located at the states of Minas Gerais and Rio de Janeiro. Herd location was divided in region 1 (north) and region 2 (south). The observed number of dairy herds in regions 1 and 2 was 42 and 70, respectively. A simple randomized sampling, stratified by region for finite population, was used to calculate the number of herds. One bulk tank milk sample was collected from 40 herds to identify *S. aureus* and *S. agalactiae* using selective media. From these herds, 16 and 24 were located in regions 1 and 2, respectively. The real prevalence of *S. aureus* and *S. agalactiae* was calculated based on the apparent prevalence, sensitivity and specificity of one bulk milk sample culture from a previous study performed in Brazil [7].

Pathogen	Statistics	Region		Total
		1*	2*	
S. aureus	N	16	24	40
	AP (CI)	0.75[a] (0.54-0.96)	0,67[a] (0.48-0.86)	0.70 (0.56-0.84)
	RP (CI)	1.00 (0.72-100)	0.89 (0.64-1.00)	0.93 (0.74-1.00)
S. agalactiae	N	16	24	40
	AP (CI)	0.38[a] (0.14-0,62)	0.21[a] (0.05-0.37)	0.28 (0.14-0.41)
	RP (CI)	0.57 (0.21-0.93)	0.31 (0.07-0.56)	0.41 (0.20-0.62)

[a]Equals letters between columns means no statistical difference (p>0.05)

*Region 1 – north; Region 2 – south; N – number of herds; AP – Apparent prevalence; RP – real prevalence; CI – confidence interval 95%.

Table 3: Apparent prevalence, real prevalence and confidence intervals of *Staphylococcus aureus* and *Streptococcus agalactiae* among dairy herds in the Minas Gerais Holstein Breeders Association, 2011-2012

The real prevalence of *S. aureus* and *S. agalactiae* was 93.0% and 41.0%, respectively. The real prevalence of *S. aureus* among herds located in region 1 and 2 was 100.0% and 89.0%, respectively. As for *S. agalactiae,* the real prevalence was 57.0% and 31.0% in regions 1 and 2, respectively (Table 3). The results demonstrated a high prevalence of *S. aureus* and *S. agalactiae* in these herds and a homogeneous distribution among herds within region 1 or 2. The adoption of control measures considering epidemiological features of each pathogen and the prevalence of infected cows within each herd should be taken into account if the objective is to

reduce the rate of new infections and infection time. In that matter, the main approach for prevention and control of *S. agalactiae* may be directed to the eradication of this pathogen through the treatment of infected cows. In contrast, control measures for *S. aureus* should be based on culling cows with chronic infections due to its difficult elimination. The high prevalence of *S. aureus* and *S. agalactiae* in these herds suggests that control measures have not been effectively adopted and the prevalence of these pathogens were homogenously distributed between the two studied regions.

Regional and herd level

Risk factors for the occurrence of new and chronic cases of subclinical mastitis in dary herds in the southern Brazil [12]

The month-to-month monitoring of SCC in cows is an important tool for decision making to prevent subclinical intramammary infection (IMI) [2,27]. The analysis of mastitis risk factors can help identifying measures to improve mammary gland health in dairy herds and such analysis is often based on a combination of diagnosis and monitoring systems [28]. Thus, the identification of risk factors associated with the occurrence of IMI can help improve programs for the prevention and control of mastitis in dairy herds [29,30]. Therefore, the study described below aimed to evaluate risk factors associated with new cases of subclinical IMI and the persistence of such infections using monthly SCC data from dairy cows in herds located in southern Brazil.

The study was conducted in the west, midwest, south, and highland regions of the state of Santa Catarina, in southern Brazil, and involved 30 dairy farms enrolled in the Dairy Herds Improvement (DHI) test of the Santa Catarina Cattle Breeders Association (ACCB, Florianopolis, Brazil), with monthly information about milk yield, milk composition, and SCC in cow composite samples.

The study involved 1,700 lactating cows. On average, dairy farms had 47.1 lactating cows and 13.3% of the herds had ≤20 cows, 36.7% had 21 to 40, 26.7% had 41 to 60, 13.3% had 61 to 80, and only 10.0% had ≥80 cows. The study used data from 11,159 DHI dairy assessment tests, occurring from December 2011 to November 2012. Each DHI test recorded information about breed, parity, days in milk (DIM), milk, SCC, and date of calving.

At the beginning of the experiment, dairy farmers received a survey questionnaire to provide information about their herd size, the infrastructure of their farm, and factors related to mastitis (techniques used in milking management and milking facilities, drugs used, and removal (culling) of animals affected by mastitis). Each farm received a technical visit to evaluate the occurrence of teat-end hyperkeratosis, udder depth and cleanliness, and to update the database containing information on lactating cows, as described below.

The severity of hyperkeratosis was evaluated using a scale from 1 to 4 (1 = teat-end without a ring; 2 = teat-end with small ring just forming; 3 = teat-end with rough ring just forming; 4 = teat-end with a well-developed rough ring) according to the methodology described by Mein *et al.* [31]. The mean hyperkeratosis score for the four teats of each cow was then calculated. Udder depth was evaluated before milking and was estimated as the distance from the udder floor to the point of hock, using a scale from 1 to 3 (1 = udder floor above the hock, 2 = udder floor at hock, 3 = udder floor below hock) as described by Coentrão *et al.* [11]. The cleanliness of the udder was also evaluated before milking, using a 1 to 4 scale (1 = totally clean, 2 = slightly dirty, 3 = mostly dirty, 4 = completely covered with dirt) using the method described by Schreiner *et al.* [32]. The infection status of each cow for subclinical mastitis during each month of the study was assessed by comparing two consecutive months, evaluating the SCC of the current month´s DHI test relative to the previous month, as described by Ruegg [33]. Infection was defined as SCC ≥200,000 cells/ mL [2,34,35]. Cows were classified as healthy, newly infected, or chronically infected as described by Schukken & Kremer and Malek & Santos [36,37].

The mean SCC score across all tested cows was 494,000 cells/mL. Given the maximum limit of 200,000 cells/mL established in this study, it was concluded that only 43.3% of the cows were in good health at any given time.

A final logistic model for the risk of new cases of subclinical mastitis, involving three groups of variables (those related to individual cow characteristics, milking management and mastitis control techniques, and farm structure) included the variables parity, teat-end hyperkeratosis, udder depth, cleanliness of the udder, and milking cows with mastitis last (Table 4). Cows with parity ≥4 (OR=1.71; $P < 0.01$) and cows with a mean score of teat-end hyperkeratosis ≥3 (OR=1.59; $P < 0.05$) were at a greater risk of new subclinical cases. Cows with extremely deep udders (udder floor below hock) were at a higher risk of new subclinical IMI compared to cows with shallow udders (udder floor above hock; $P < 0.001$), or cows with udder floor at hock ($P < 0.01$). Cows with very dirty udders and dairy farms that did not leave infectec animals to milk last also had a higher risk of new cases of subclinical mastitis ($P < 0.01$).

			95% CI OR	
Explanatory variable	Category	OR	Below	Above
Parity	11	0.95[2]	0.65	1.38
	2			
	3	1.34[2]	0.92	1.96
	>4	1.65*	1.11	2.44

Hyperkeratosis	1 to 31			
	3 to 4	1.61*	1.12	2.31
Udder depth	Above the hock1			
	On the hock	1.70**	1.18	2.45
	Below the hock	2.46***	1.67	3.62
Udder cleanliness	Clean1	1.33[2]		
	Slightly dirty		0.98	1.81
	Very dirty	1.55**	1.11	2.14
Cows with mastitis are milked last	Yes1			
	No	1.55**	1.14	2.09
P-value of the model			<0.001	
Adjustment of the model by the test of Hosmer and Lemeshow			0.826	

[1]Reference category
[2]Not significant.
*P < 0.05; **P < 0.01; ***P < 0.001.
Table 4: Odds ratios (OR) and confidence intervals of new intramammary infections (IMI) based on the final logistic regression model

The final logistic regression model to determine the risk of developing chronic subclinical infection relative to the new cases of subclinical mastitis was composed by maintenance of milking equipment, DIM, and udder depth (Table 5). Cows with deep udders and dairy farms that performed only sporadic maintenance of the milking equipment had a higher risk of chronic infections ($P < 0.001$). In addition, cows with DIM≥100 were at a higher risk of chronic infection ($P < 0.001$). Strategies for controlling and monitoring mammary gland health in dairy cows are mainly designed to reduce the number of new infections, to eliminate established infections, and to decrease the duration of infections by using recommended techniques.

			95% CI OR	
Explanatory variable	Category	OR	Below	Above
Maintenance of milking equipment	Periodic[1]			
	Sporadic	2.17***	1.62	2.89
Days in milk	Up to 100[1]			
	101 to 200	2.70***	1.90	3.83
	201 to 300	5.88***	3.88	8.87
	Over 300	4.62***	3.04	7.04
Udder depth	Above the hock[1]			
	On the hock	1.68**	1.18	2.37
	Below the hock	1.65**	1.17	2.34
P-value of the model			<0.001	
Adjustment of the model by the test of Hosmer and Lemeshow			0.889	

[1]Reference category.
**P < 0.01; ***P < 0.001.
Table 5: Odds ratios (OR) and confidence intervals of chronic intramammary infections (IMI) based on the final logistic regression model

Cows with a greater number of lactations (parity ≥4) were at a higher risk of new cases of subclinical mastitis, a finding that is in agreement with results of previous studies and perhaps occur simply because of the prolonged exposure to infections that come with age (Table 4) [38-42]. It is noteworthy that animals with a higher number of calvings tend to have more injuries that are permanent in the mammary gland during lactation, resulting in a greater number of prolonged infections [43]. Udder cleanliness did not affect the occurrence of chronic mastitis, indicating that environmental conditions are more related to the initial contamination of the mammary gland [32].

Dairy farms that did not sort infected animals for milking at last had a higher risk of new IMI cases (Table 4). Accordingly, milking infected animals after healthy ones reduced the incidence of IMI. This management technique is designed to prevent the contamination of uninfected animals and these data demonstrates its effectiveness [33,44,45]. The number of days a cow had been in milk (DIM) affected the risk of chronic subclinical infection, especially for cows with DIM ≥200 d, similar to findings of previous reports [46-48] (Table 5). According to Hagnestam-Nielsen *et al.*, the increase in SCC in cows at the end of lactation is probably due to an increased cellular response to residual injuries from previous infections that results in major damage to the mammary tissue [49]. A higher risk of new cases of subclinical mastitis in the presence of teat-end hyperkeratosis was also observed in our study (Table 4). Inadequate pulsation of the milking machine, removal of teat cups without removing the transfer vacuum, and

overmilking are among the possible causes of hyperkeratosis [50-52]. In fact, we observed that the lack of regular maintenance of the milking equipment increased the risk of chronic cases of IMI (Table 5).

Correct milking management is important for the control of mastitis and for the production of high-quality milk. Moreover, appropriate cleaning of teats before milking makes it harder for putative infectious agents to penetrate into the gland [33]. Similarly, Elmoslemany *et al.* [53] reported that premilking teat disinfection with subsequent drying of the teats with paper towels reduced bacterial count in milk, whereas postmilking teat disinfection was a management technique widely known for the prevention of mastitis [44,54]. The main risk factors associated with the occurrence of new cases of subclinical mastitis in dairy cows were advanced age, deep udders, dirty udders, presence of teat-end hyperkeratosis, and milking healthy cows prior to cows affected by mastitis. Factors such as DIM, deep udders, and farms that perform only sporadic maintenance of the milking equipment were also associated with a higher risk of chronic subclinical mastitis.

Herd and animal level

Reduction in *Streptococcus agalactiae* prevalence by intramammary antibiotic treatment in a Holstein dairy herd under tropical conditions [9]

S. agalactiae and *S. aureus* are contagious pathogens and considered as major mastitis pathogens, because of their extensive effects on milk quality, production, and somatic cell count (SCC) [55]. The primary method of spreading these pathogens is cow-to-cow, so the focus of prevention should be to reduce or eliminate herd infection [55]. In herds where subclinical mastitis infections are from these contagious pathogens, the eradication of *S. agalactiae* and prevention and control of *S. aureus* must be the main objectives. Eradication of *S. agalactiae* can be completed rapidly through a culture followed by treatment program with minimal culling. For *S. aureus*, treatment success, particularly during lactation, is often disappointing and depends on cow, pathogen, and treatment-related factors [55]. Culling animals with chronic infection by *S. aureus* is thus the most recommended strategy. Therefore, the microbiological diagnosis of mastitis is fundamental for the adoption of specific control measures, separation and culling of chronically infected animals, evaluation of treatment efficacy, and the establishment of antimicrobial susceptibility panels [56]. This study evaluated the efficacy of intrammamary antibiotic treatment in a Holstein dairy herd in the process of controlling *S. aureus* and *S. agalactiae* under tropical conditions.

The study was conducted in dairy cattle herds located in the Atlantic forest region, Minas Gerais, Brazil. The herds are registered in the state of Minas Gerais Holstein Breeders Association, which has 112 herds. These herds have some characteristics in common such as breed, nutritional and reproductive management, mechanical milking procedures, and record keeping (productive, economic and mammary gland health indices). The studied herd had an average of 142 lactating Holstein cows/month and milk yield of 8,050 Kg over a 305 days lactation. Lactating cows were subjected to official milk production control through a monthly assessment of milk production and collection of milk samples to determine composition (fat, protein, lactose and total solids) and somatic cells count. Milk samples from the herd (bulk tank) were collected directly into vials containing a preservative (Bronopol®, D&F Control Systems Inc., US) and submitted to somatic cell counting (SCC). Analyses of SCC were performed by the Milk Quality Laboratory, at Embrapa Dairy Cattle, using an automated flow cytometer (Somacount 300, Bentley Instruments Inc., US) [57].

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. Milk samples were taken from all lactating animals at a given timepoint, excluding those presenting clinical mastitis at the time of collection and those being treated with antibiotics. The reason for sampling only lactating cows was an attempt to identify those infected by *Streptococcus agalactiae* and, if that was the case, begin a treatment in all mammary quarters with intrammamary antibiotics. Secondly, we sought to identify lactating cows chronically infected by *S. aureus* for possible culling. The intrammamary antibiotics used in cows infected by *S. agalactiae* were cloxacillin (200mg) and ampicillin (75 mg) (Bovigam®L, Bayer S.A., São Paulo, Brazil) administered three times at 12-hour intervals. A milk withdrawal period of 72 hours was adopted following the manufacturer's instructions. Cows with more than two successive *S. aureus* isolations were selected for culling. The estimated prevalence of *S. agalactiae* and *S. aureus* among lactating cows was calculated dividing the number of infected cows for each pathogen by the total number of lactating cows in the herd.

The milk sample collection and transport procedures were according to the National Mastitis Council guidelines [58,59]. The mastitis-causing agents were identified in the Milk Microbiology Laboratory, at Embrapa Dairy Cattle. Based on the microbiological and biochemical test results, cows infected with *S. agalactiae* received the intramammary treatment mentioned previously. The percentages of infected cows with *S. agalactiae* in January 2012 and January 2013 were 61.6% and 2.2%, respectively (Table 6). In the same months, the percentages of cows infected with *S. aureus* were 28.3% and 19.4%, respectively (Table 6). The consequence of the treatment of cows infected with *S. agalactiae* was the reduction of approximately 60% in the prevalence of infected cows. The lowest S. agalactiae prevalence (0.7%) was observed in November, 2012, meaning one infected cow. The reduction in prevalence of *S. agalactiae* was continuous from January 2012 to March 2012. After this period, the prevalence oscillated between 0.7% and 6.0%.

Month/Year	Lactating cows (n)	Results of microbiological test			
		Staphylococcus aureus		*Streptococcus agalactiae*	
		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

Table 6: Results of microbiological testing according to contagious mastitis pathogens in one dairy herd from January, 2012 to January, 2013

We performed intrammamary antibiotic treatments in lactating cows infected by *S. agalactiae* after we received results of microbiological tests, i.e., approximately 5 to 7 days after milk sample collection. The time between sample collection and microbiological tests results can be associated with the oscillation observed on the prevalence of *S. agalacitae* among lactating cows, because during this interval infected cows have not been treated and were therefore a source of infection to non-infected cows. The reduction in *S. agalactiae* prevalence was due to 161 intramammary antibiotic treatments of individual cows diagnosed as infected during lactation within the study period.

S. agalactiae is a pathogen of the bovine mammary gland and rapid and successful eradication of this microorganism from the herd may be achieved by intrammamary treatment of infected cows [55]. A study on mastitis contagious pathogens was carried out in the herds of the State of Minas Gerais Holstein Breeders Association and a mean prevalence of 40% was observed for *S. agalactiae* among all herds [8]. The high prevalence *S. agalactiae* in these herds suggests that control measures have not been adopted properly [8]. On the other hand, a reduction of approximately 9% in the prevalence of *S. aureus* was detected during the study period. Prevalence of *S. aureus* oscillated between January to April, 2012, but after this period decreased continuously. Nine cows with chronic *S. aureus* infections were culled from January, 2012 to January, 2013 and this procedure was associated with the decrease in *S. aureus* prevalence. The success in treatment of infected animals is more challenging because *S. aureus* is an intracellular pathogen [55]. Thus, culling chronically infected cows is the main strategy indicated to control the infection by this pathogen within a herd [55]. The impact on the reduction of the herd SCC from1, 175,000 cells/mL to 899,000 cells/mL was probably due to a decrease in the percentage of animals infected with both pathogens. Before intrammamary antibiotic treatments, the percentage of cows with SCC ≤200,000 cells/mL, between 200,000 and 400,000 cells/mL and ≥400,000 cells/mL were 85.5%, 12.0% and 2.5%, respectively. The consequence of the treatment of *S. agalactiae*-infected cows was the reduction in the percentage of cows with SCC ≥400,000 cells/mL from 67.9% to 56.5% and the increase in the percentage of cows with SCC ≤200,000 cells/mL from 21.4% to 30.6%. Well managed herds should have a prevalence of chronic infections (individual cows repeatedly ≥200,000 cells/mL) of less than 5% and the incidence of new intramammary infections (cows above the 200,000 threshold) of less than 5% in a monthly basis [55]. Moreover, the percentage of cows above the SCC cut-off limit (200,000 cells/mL) should be less than 20% [36]. According to Philpot & Nickerson, cows with SCC ≥400,000 cells/mL had a decrease in milk production of approximately 538 to 898 kg per lactation [60]. This information was confirmed in Brazil by Cunha *et al.* [61]. In that study, Holstein animals raised under tropical conditions produced less milk as SCC increased. Notably, the mean milk production per cow over a 305-days lactation increased from 7,763 to 8,040 kg. This increase in production may be due to a decrease in the percentage of cows infected with contagious mastitis pathogens, especially *S. agalactiae*, rather than the reduction of cows infected with *S. aureus*.

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

Herd and animal level

Estimate of the economic impact of mastitis: a case study in a Holstein dairy herd under tropical conditions [16].

The main components of the economic impact of mastitis are related to the decrease in milk production due to clinical and subclinical cases, milk withdrawal, drug costs for treatment of clinical disease, labor costs related to the treatment of clinical cases, the decrease in milk sale price and the culling of animals [15]. The estimated weight (percent) of each component on total economic impact of mastitis at the herd level combined with clinical and subclinical mastitis indicators can assist in a decision-making

process focused on the economic aspects of the disease and define priorities in terms of the cost-benefit of control procedures [62]. Only a few studies, however, have been conducted in Brazil to evaluate the total economic impact of mastitis in dairy cattle herds. Therefore, the study had the objective of assessing the economic impact of mastitis and the weight of specific components of this impact on a Holstein dairy herd in the process of controlling *Staphylococcus aureus* and *Streptococcus agalactiae* occurrence under tropical conditions.

The study was performed in a dairy herd located in Zona da Mata, State of Minas Gerais, Brazil. The enrolled herd had an average of 142 lactating Holstein cows/year and milk production of 8,050 Kg of milk in 305 days of lactation. The estimate of the economic impact of mastitis at the herd level was performed using the "Mastitis calculation tool at farm level" method [15]. Three estimates of the economic impact of mastitis at the herd level were performed for the period from February, 2011 to January, 2013. Production and economic indices from February, 2011 to January, 2012 were used in Estimate 1. In Estimate 1, clinical and subclinical mastitis indices and the percentage of cows culled due to mastitis were the ones described by Schukken & Kremer and were classified as the ideal indices. In Estimate 2, the production, economic, and clinical and subclinical mastitis real indices from February, 2011 to January, 2012 were considered [36]. Real indices were those recorded at the farm and by the Minas Gerais Holstein Breeders Association database. Estimate 3 considered production, economic and clinical and subclinical mastitis real indices from February, 2012 to January, 2013. The components evaluated in the economic impact of mastitis were the reduction in milk production due to clinical and subclinical mastitis, milk withdrawal, drug costs for treatment, labor and cow culling. The estimates generated in the study were associated to the economic impact of mastitis at the herd level during the two-year period.

Considering clinical and subclinical mastitis ideal indices and the production and economic indices (Estimate 1), the total economic impact of mastitis in the studied herd was US$19,132.35. The three main components of the economic impact were cow culling, reduction in milk production due to subclinical mastitis, and reduction in milk production due to clinical mastitis, which corresponded to 39.4%, 32.3% and 18.2% of the total economic impact, respectively. Ideally, culling of approximately 15% of the animals with chronic mastitis is recommended to reduce the sources of infection in the herd, especially in the case of contagious mastitis [14,15,19]. For the ideal situation, we considered culling five cows with persistent *S. aureus* infection and represented 3% of the total lactating cows. We took this approach because cows with chronic and subclinical infections for longer periods are predisposed to manifest clinical disease.

Milk production losses due to clinical mastitis accounted for 18.2% of the total economic impact considering the ideal standard of 25 cases per 100 cows [36]. The weights of economic impact due to milk withdrawal (7.2%), drug costs (2.9%) and labor (0.03%) accounted together for approximately 10% of the total impact. In a study conducted by Lopes Junior *et al*. to simulate, analyze and quantify the impact of clinical mastitis, within dairy herds in which the number of clinical mastitis cases per year raised from 1% to 15%, the economic impact of milk withdrawal increased from 13.5% to 52.9%. However, in that same study, the economic impact was simulated only for clinical mastitis and was estimated as the total losses plus the expenses for prevention and treatment of clinical cases [4].

Considering the real milk production, economic, and clinical and subclinical mastitis indices, Estimate 2 indicated a total economic impact of US$61,623.13 for the period from February, 2011 to January, 2012 (Table 7). The difference between an ideal situation and the real situation for clinical and subclinical mastitis indices was US$42,490.72. The weight of the components with an economic impact was different from Estimate 1, with the reduction in milk production due to subclinical mastitis (42.2%) and clinical mastitis (35.5%) and milk withdrawal (14.0%) acting as the three main components. Drug costs (5.6%), cow culling (2.4%) and labor costs (0.04%) together accounted for approximately 8.0% of the total economic impact of mastitis at the herd level.

Economic impact components	Economic impact of mastitis US$/year (%)		
	Estimate 1	Estimate 2	Estimate 3
Decrease in milk production - clinical mastitis	3,485.04 (18.2)	21,886.06 (35.5)	31,761.97 (34.7)
Decrease in milk production - subclinical mastitis	6,186.65 (32. 3)	26,157.33 (42.2)	18,537.44 (20.2)
Milk withdrawal	1,371.16 (7.2)	8,610.91 (14.0)	20,117.49 (22.0)
Drug costs for treatment	547.00 (2.9)	3,435.16 (5.6)	8,999.60 (9.8)
Labor	5.00 (0.03)	26.17 (0.04)	76.20 (0.08)
Cow culling	7,537.5 (39.4)	1,507.50 (2.4)	12,060.00 (13.2)
Total	**19,132.35**	**61,623.13**	**91,552.69**

Estimate 1: real production and economic indices for the period from Feb/2011 to Jan/2012 and the ideal clinical and subclinical mastitis indices (Schukken and Kremer, 1996); Estimate 2: real production, economic, and clinical and subclinical mastitis indices for the period from Feb/2011 to Jan/2012; Estimate 3: real production, economic, and clinical and subclinical mastitis indices for the period from Feb/2012 to Jan/2013.
Table 7: Economic impact of mastitis with ideal mammary gland health data in the studied situations

In Estimate 1, the weight of culling animals due to mastitis was approximately 16-fold greater than the weight in Estimate 2, demonstrating that culling cows, especially those with chronic infections, was not a procedure adopted during the respective study period because only one cow was culled. By comparing Estimates 1 and 2, we observed that the major economic impact of mastitis resulted from cow culling, representing 50% of the total economic impact in Estimate 1, whereas this component accounted for

only 3.5% of the total economic impact in Estimate 2. In an ideal situation, culling of approximately 15% of the animals with chronic mastitis is recommended to eliminate animals that are considered reservoirs of pathogenic agents and sources of infection for their healthy herdmates [14,15,19].

The impacts of the decrease in milk production due to clinical and subclinical mastitis were 17.3% and 9.9% higher than the impacts in Estimate 1. The weight of the impact of milk production reduction due to clinical cases was approximately two-fold greater than the weight in Estimate 1. This difference was probably caused by the actual 157 clinical cases per 100 cows compared to 25 clinical cases per 100 cows used as ideal index in Estimate 1. Similarly, milk withdrawal (14.0%) had a weight approximately two-fold higher than the weight in Estimate 1 due to the high incidence of clinical mastitis observed.

For the reduction in milk production due to subclinical mastitis, the weight was greater in Estimate 2 than in Estimate 1. In lactating cows, an increase in SCC was associated with decreased milk production following the formulas used in the "Mastitis calculation tool at farm level" tool [15]. The reduction in milk production due to clinical mastitis cases was the component with the greatest weight (42.2%) in the total economic impact of mastitis due to the large percentage of cows with SCC ≥400,000 cells/mL (68.0%). It is noteworthy that the greatest weight for the increase of total economic impact of mastitis in Estimate 2 was obtained by losses in production due to the occurrence of clinical and subclinical mastitis in the herd, representing approximately 78.0% of the total impact.

Although a reduction in milk yield had a large weight on the economic impact of mastitis, the magnitude of this reduction was often neglected by the herd owner, who had more difficulties in understanding this phenomenon rather than the direct losses represented by milk withdrawal, cow culling and the purchase of supplies, such as teat disinfectants and antibiotics.

Considering the real production, economic, clinical and subclinical mastitis indices, Estimate 3 revealed a total economic impact of US$91,552.69 for the period from February, 2012 to January, 2013. Twelve months after the first real estimate, an increase of US$29,929.56 in the economic impact of mastitis was observed at the herd level. The reduction in milk production continued to be the component with the greatest weight in total economic impact (54.9%), with losses in production due to clinical and subclinical mastitis accounting for 34.7% and 20.2% of the total input, respectively. Milk withdrawal represented 22.0% of the economic impact, whereas cow culling and drug costs accounted for 13.2% and 9.8%, respectively.

Estimate 3 results demonstrated an overall increase in the total economic impact of mastitis as a function of the 161 intramammary antibiotic treatments of cows infected with *S. agalactiae* during lactation, with drug costs and milk withdrawal substantially larger than Estimate 2. Additionally, nine cows chronically infected with *S. aureus* were culled and contributed to the increase in total costs of mastitis in Estimate 3. Nonetheless, we observed a drastic decrease in the weight of the impact of the reduction in milk production due to subclinical mastitis. This effect was primarily due to a decrease in the prevalence of *S. agalactiae* in the herd, which was the main pathogen responsible for high SCC in lactating cows and, consequently, the herd [19]. Notably, the average milk yield per cow over a 305 days lactation increased from 7,763 kg to 8,040 kg during the period considered for Estimate 3. This increase in production might have been due to a reduction in the percentage of cows infected with contagious mastitis pathogens, especially *S. agalactiae*, rather than a consequence of the reduction in the number of cows infected with *S. aureus*.

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