Preventive Veterinary Medicine 121 (2015) 297–305

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle

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ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 17 July 2015
Accepted 3 August 2015

Keywords:
Clinical mastitis
Risk factor
Brazilian dairy industry
Pathogen

ABSTRACT

Information related to mastitis risk factors is useful for the design and implementation of clinical mastitis (CM) control programs. The first objective of our study was to model the risk of CM under Brazilian conditions, using cow-specific risk factors. Our second objective was to explore which risk factors were associated with the occurrence of the most common pathogens involved in Brazilian CM infections. The analyses were based on 65 months of data from 9,789 dairy cows and 12,464 CM cases. Cow-specific risk factors that could easily be measured in standard Brazilian dairy farms were used in the statistical analyses, which included logistic regression and multinomial logistic regression. The first month of lactation, high somatic cell count, rainy season and history of clinical mastitis cases were factors associated with CM for both primiparous and multiparous cows. In addition, parity and breed were also associated risk factors for multiparous cows. Of all CM cases, 54% showed positive bacteriological culturing results from which 57% were classified as environmental pathogens, with a large percentage of coliforms (35%). Coagulase-negative *Staphylococcus* (16%), *Streptococcus uberis* (9%), *Streptococcus agalactiae* (7%) and other *Streptococci* (9%) were also common pathogens. Among the pathogens analyzed, the association of cow-specific risk factors, such as Zebu breed (OR = 5.84, 95%CI 3.77–10.77) and accumulated history of SCC (1.76, 95%CI 1.37–2.27), was different for CM caused by Coagulase-negative *Staphylococcus* and *S. agalactiae* in comparison to CM caused by coliforms. Our results suggest that CM control programs in Brazil should specially consider the recent history of clinical mastitis cases and the beginning of the lactations, mainly during the rainy season as important risk factor for mastitis.

1. Introduction

Brazil is a tropical country that produced 35 billion liters of milk in 2013 (IBGE, 2014). It is the fifth largest milk producing country worldwide and has considerable potential to increase milk production, given its large territory and favorable conditions for agricultural activities (Martinelli et al., 2010).

Mastitis is generally regarded as one of the most costly diseases in dairy herds (e.g. Huijps et al., 2008; Cha et al., 2011; Hogeveen et al., 2011). The high incidence of clinical mastitis (CM) in Brazil is considered to be one of the greatest challenges to the Brazilian dairy industry (Oliveira et al., 2009). It is important to reduce mastitis incidence to improve productivity and milk quality (e.g. Halasa et al., 2007; Cha et al., 2011; Hogeveen et al., 2011).

Mastitis control programs have recently been developed in Brazil, and the demand for research on mastitis risk factors is growing with the implementation of these programs. Only a few studies on mastitis risk factors in Brazil have been published and these studies only consider a limited number of risk factors (Prestes et al., 2002; De Oliveira et al., 2010; Lima et al., 2013).

The incidence of CM is associated with many risk factors. The sampling unit in risk factor studies can vary from quarter level to herd level (Leelahapongsathon, 2014). Quarter-specific risk factors are responsible for the difference in CM occurrence in different quarters of the same animal. They include teat position, distance from teat to floor, presence of previous hyperkeratosis and bacterial infection (e.g. Neijenhuis et al., 2001; Green et al., 2007; Leelahapongsathon et al., 2014). Cow-specific risk factors are related to the difference in CM incidence among cows. Parity, month of lactation, season of the year, somatic cell count (SCC) in previous lactation and CM history are the cow-specific risk

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http://dx.doi.org/10.1016/j.prevetmed.2015.08.001
0167-5877/

factors, which are currently known (e.g. Olde Riekerink et al., 2008; Steeneveld et al., 2008; Breen et al., 2009). Herd-specific risk factors are involved in the differences of CM incidence among dairy farms and are related to deficiencies in farm management, such as a lack of dry cow therapy, milking machines with inadequate suction pressure and common tissues for udder preparation (e.g. Barkema et al., 1999; Peeler et al., 2000; Nyman et al., 2007).

The efficiency of CM control can be improved by using information about cow-specific risk factors. This information allows a farmer to identify the cows that have a higher risk of CM and to subsequently provide a higher level of care for these cows. The husbandry conditions in Brazil differ from the conditions in countries in Europe and North America, where most of the cow-specific risk factors studies have been conducted. One example is the presence of just two seasons in Brazil (Embrapa, 2012). Despite season is not a cow factor we expect an interaction of it with cow-specific risk factors, such as SCC. Summer is the rainy season and winter is the dry season, and the highest incidence of mastitis occurs in summer (Costa et al., 1998). The presence of crossbred European-Zebu cows in Brazilians herds is another example of a factor that might give a different set of risk factors for CM.

In addition to cow-specific risk factors, knowledge of the pathogens involved in CM is also useful to improve the efficiency of CM control measures (Steeneveld et al., 2008). Approximately 95% of mastitis infections in Brazil are caused by *Streptococcus agalactiae*, *Staphylococcus aureus*, *Streptococcus uberis* and Coliforms (e.g. Brito and Sales, 2007; Santos et al., 2007). Coagulase-negative *Staphylococcus* (CNS) species are the organisms most frequently isolated from bovine milk samples worldwide. CNS are a part of the normal teat skin microbiota and can also cause mastitis infections (e.g. Piessens et al., 2011; Supré et al., 2011).

The current lack of information about risk factors for CM in Brazilian herds and the usefulness of this knowledge to design and implement mastitis control programs provided the motivation for this study. The first objective of this study was to model the risk of having CM under Brazilian conditions, using all feasible cow-specific risk factors and data from 9,789 Brazilian dairy cows. The second objective of the study was to identify and quantify risk factors related to the occurrence of the most common pathogens involved in CM infections in Brazilian farms.

2. Materials and methods

2.1. Herd and data description

Data from eight dairy herds located in Minas Gerais, Brazil were used in this longitudinal retrospective study, which covered a period of 65 months from January 2009 to May 2014. These herds were chosen because of the availability and quality of data; farmers, were literate and authorized the use of their data, which was provided by a farm management enterprise.

Milking was done mechanically twice a day on all farms. Milking personnel was familiar with the symptoms of clinical mastitis (warm, swollen udder and/or changes in milk). They were instructed by veterinarians to register all occurrences of CM in an internal database and to collect milk samples from quarters with CM and send them for bacteriological analysis to certified laboratories. Bacteriological culturing was performed according to the standards of the International Dairy Federation (1995).

The majority of the cows (84.5%) were crossbreds, i.e. Zebu breeds (*Bos taurus indicus*) crossed with European breeds (*Bos taurus taurus*). In total, 154 levels of combinations of Zebu-European crossbreds were present. Of all cows, 15.5% of cows were pure Holstein and 0.02% pure Dairy Gir breed (*Bos taurus indicus*). Cows were kept on pasture during the entire year. In addition to grass, the diet composition included corn, soybean meal, citrus pulp, barley, minerals and forage. The forage fed to cows varied within and among farms, according to soil quality, seasons and differences in nutritional value of the grass. The fluctuation in the number of dairy cows and the average milk production per cow during the study period is shown in Table 1.

Two sets of data were available. The first dataset contained monthly records of milk production (MPR) for all cows and the second dataset contained data on the recorded cases of CM. In both datasets, each record contained data for a single monitoring event. The MPR for all cows was registered on a monthly basis. These records contained the identification number (ID) of the farm, cow ID, recording date, breed, milk production, days in milk (DIM) on the day of the record, parity, calving and SCC. Monitoring of cows with CM occurred daily during the CM period. The CM monitoring records contained the recording date, DIM on the day of the record, antibiotic base applied and bacteriological culture result, if available.

2.2. Data preparation

The CM monitoring dataset was merged with the MPR dataset using recording date, farm ID and animal ID. The outcome of interest was whether or not mastitis occurred based on the monthly records. The merged dataset contained 168,717 records covering 30,970 lactations from 9,912 dairy cows and 31,755 CM cases. Lactations that were not recorded from calving onwards were excluded ($n = 247$) to avoid the inclusion of CM cases that started before the study period as in accordance with Steeneveld et al. (2008). Records with calving intervals smaller than 320 days or greater than 600 days were excluded ($n = 228$), as well as records with no milk production information ($n = 76$). The month in which the mastitis case started for each cow was considered as the month of CM occurrence. The outcome of interest was the monthly records when mastitis occurred or not occurred. If the interval (period in which no clinical signs occurred) between two CM records of the same cow was greater than 14 days, it was defined to be a new CM case. CM cases were registered at cow level because the quarter level was not recorded. As a consequence, if CM occurred in different quarters during an interval shorter than or equal to 14 days, this was considered one CM case. Given this definition, 19,290 CM observations were considered not to be new CM cases. Following these exclusions, the final dataset consisted of 163,208 records covering 30,419 lactations from 9,789 dairy cows with 11,914 CM cases. Of the 11,914 CM cases, 24.37% had registered bacterial culture results. Potential risk factors and their respective levels were defined based on literature and the authors' expertise (Table 2). Cow-specific risk factors that could easily be measured in standard Brazilian dairy farms were used in the statistical analyses. Despite the greater value of including continuous variables, we categorized DIM because the estimate of DIM was nonlinearly distributed over the range of DIM. Parity and DIM were categorized in four and seven categories respectively, creating the variables PAR and months in milk (MIL) in accordance with Steeneveld et al. (2008), Breen et al. (2009) and Leelahapongsathon et al. (2014). Two season categories, rainy (1) and dry (0), were considered (Fonseca et al., 2005). To explore the influence of crossbred European-Zebu cows, a categorical variable 'Breed' was created. Cows with more than 15% of Zebu breed in their genetic constitution were considered *Bos taurus indicus* (type 1) and all others *Bos taurus taurus* (type 0). The variable SCC1 represents the natural logarithm of the SCC in the previous month of lactation. SCC2 is the geometric mean of all natural logarithm of SCCs of all months prior to the previous month, including previous lactations, if available. The influence of the CM history, in accordance with Steeneveld et al. (2008), was analyzed using the variables MAST1 and MAST2. MAST1 represented the most recent history and consisted of the number of CM cases in the previous

Table 1
Number of lactating cows (N) and average milk production per cow per year (liters) per farm from 2009 to 2014 using data of 8 Brazilian dairy herds.

Farm	2009		2010		2011		2012		2013		2014[a]	
	N	Milk	*N*	Milk	*N*	Milk	*N*	Milk	*N*	Milk	*N*	Milk
1	258	6,264	286	6,021	249	5,446	242	5157	271	5,694	236	6,021
2	196	9,051	206	9,184	209	9,526	232	8052	212	8,599	205	8,800
3	1,033	7,486	1,255	7,128	1,228	6,431	1,095	6263	879	7,058	663	5,995
4	423	7,475	452	6,697	441	7,379	450	6967	451	7,000	437	6,652
5	81	7,728	180	7,622	525	7,690	397	7108	488	10,010	410	8,744
6	322	5,634	312	4,603	448	5,507	467	5904	585	6,200	360	5,756
7	372	6,621	315	6,816	394	7,119	439	7109	454	7,817	307	7,332
8	629	7,515	679	7,306	678	7,079	400	6,598	271	7,192	157	6,975
Total	3,314	7,217	3,685	6,921	4,172	6,858	3,722	6,569	3,611	7,405	2,775	6,887

[a] Milk production for 2014 was estimated considering the average milk production of the first 4 months of this year.

month, with a value of 0, 1 or 2 cases. MAST2 represented the older history and consisted of the total number of mastitis cases accumulated in all months prior to the previous month, including previous lactations, if available. MAST2 was reclassified in five categories (0, 1, 2, 3 and 4 or more CM cases). The variable Year corresponded to the year in which each observation was recorded and was included to explore whether the incidence of CM differed across years.

Heifers and older animals differ regarding physiological and management factors, including that heifers are commencing lactation for the first time and are still growing. These differences may affect the occurrence of CM. For example, the incidence of CM in heifers is higher in the first few days postpartum than in multiparous cows (Barkema et al., 1998; McDougall et al., 2007). We therefore analyzed the risk factors for CM separately for heifers and multiparous cows expecting to find different risk factors or different effects of risk factors in both groups.

The cases of CM with bacterial culture results were classified in four categories based on which pathogen was isolated: Coliforms, CNS, *S. uberis* and *S. agalactiae* respectively. Records with missing culture results and cultures that resulted in no growth, growth of other pathogens or more than one pathogen in the same sample, were excluded from the analysis. Only the first culture result of

Table 2
Summary of variables with their abbreviations and the levels used in the mastitis risk factors analysis using data of 8 Brazilian dairy herds.

Variable	Abbreviation	Levels
Dependent variables		
Presence of clinical mastitis	CM	0 = no; 1 = yes
Pathogens involved	PATH	1 = Coliforms; 2 = coagulase-negative *Staphylococcus*; 3 = *Streptococcus uberis*; 4 = *Streptococcus agalactiae*
Independent variables		
Parity	PAR	1 = Parity 1; 2 = Parity 2; 3 = Parity 3; 4 = Parity ≥4
Months in lactation	MIL	1 = 1–30 days in milk (DIM); 2 = 31–60 DIM; 3 = 61–90 DIM; 4 = 91–120 DIM; 5 = 121–150 DIM; 6 = 151–180 DIM; 7 = >180 DIM
Season	SEA	1 = Rainy season (October–March); 0 = Dry season (April–September)
Breed	–	1 = Equal or more than 15% of zebu breeds (*Bos taurus indicus* type); 0 = Less than 15% of zebu breeds (*Bos taurus taurus* type)
Natural logarithm of SCC in previous month	SCC1	Continuous variable Range: 6.90–16.76 Mean: 11.88 Median: 11.89
Geometric mean of all available SCC of all months prior to the previous month	SCC2	Continuous variable Range: 8.15–15.21 Mean: 12.38 Median:12.80
Accumulated CM cases in previous month of lactation	MAST1	0,1, 2
Accumulated CM cases in all previous lactations excluding previous month	MAST2	0,1, 2, 3, ≥4
Year	–	2009–2014

Table 3
Description of the subset, number of lactations and CM cases, and the dependent and independent variables for each subset of data used in the mastitis risk factors analysis using data of 8 Brazilian dairy herds.

Subset	Description	Lactations (*N*)	CM cases (*N*)	Dependent variable[a]	Independent variables[b]
1	Primiparous data	9,294	3,632	CM	MIL, SEA, Breed, SCC1, MAST1, MAST2, Year
2	Multiparous data	21,425	8,832	CM	PAR, MIL, SEA, Breed, SCC1, SCC2, MAST1, MAST2, Year
3	Data on CM in cows with known pathogens	–	1,070	PATH	PAR, MIL, Breed, SCC1, SCC2, SEA, Year

[a] CM = Presence of CM: Yes (1) or No (0); PATH = Pathogens: Coliforms (1), coagulase-negative *Staphylococcus* (2), *Streptococcus uberis* (3) or *Streptococcus agalactiae* (4).
[b] SEA = Rainy season (1) and dry season (0); Breed = *Bos taurus indicus* type (1) and *Bos taurus taurus* type (0); PAR = Parity (1,2,3 or ≥ 4); MIL = Months in lactation (1,2,3,4,5,6 or ≥7); SCC1 = Natural logarithm of SCC in the previous month; SCC2 = Geometric mean of all available SCC of all months prior to the previous month; MAST1 = Number of CM cases accumulated in previous month (0, 1 or 2); MAST2 = Number of all previous CM cases accumulated (0,1, 2, 3 or ≥4) prior to the previous month.

each cow was kept to avoid cow-clustering and 490 observations were excluded for this reason, leaving 580CM cases with positive culture results.

2.3. Statistical analysis

All statistical analyses were carried out using R version 3.1.1 (R Core Team, 2014). First, descriptive analyses were performed. Data were analyzed in 3 subsets: risk factors for CM in heifers, risk factors for CM in multiparous cows and factors related to pathogen culture results (Table 3).

Univariable analysis between each hypothesized factor and the risk of CM infection (subsets 1 and 2) was performed using logistic regression. The logistic models were fitted with the 'lme4' package (Bates et al., 2012), binomial variance and logit link. Due to the repeated measurements per farm and per animal, the models were nested, including farm and animal as random effects. Analyses of the culture results, subset 3, were carried out using multinomial logistic regression ('nnet' package; Venables and Ripley, (2002)). Only farm was included as a random effect in the multinomial models, as we did not consider repeated measurements per cow. Year was included as a fixed effect in both, logistic and multinomial models.

Factors with a trend toward significance ($p < 0.25$) were initially considered for inclusion in the multivariable analysis. Backward stepwise logistic regression analysis (Hosmer et al., 1997), using 'Bound Optimization by Quadratic Approximation' (BOBYQA - Powell, 2009) as an optimizer, was applied to fit the subsequent multilevel mixed effects models. At first a full model was run and only variables with $p < 0.05$ in the likelihood ratio test were retained. Biologically plausible interactions among the main factors were also tested and retained in the final stage if significant ($p < 0.05$). To improve the fit of the models, the variables Parity, MIL and MAST2 were re-categorized during this stage. Confounding was checked by re-adding, one by one, the variables removed in the stepwise backward procedure. A variable was considered a confounder if its removal made the regression coefficients of the remaining variables showed a relative change >25% or in case the regression coefficient ranged between −0.4 and 0.4, if an absolute change >0.1 was observed in accordance with Noordhuizen et al. (2001). The fit of the final logistic regression models was assessed by deviance residuals evaluation (Davison and Snell, 1991). Model results are presented as odds ratios (OR) along with their 95% confidence interval (CI).

3. Results

3.1. CM in heifers

The average incidence of new CM cases in heifers was approximately 27% per year, excluding repeated cases. All risk factors analyzed presented a trend toward significance ($p < 0.25$) in the univariable analyses and were therefore included in the multivariable analysis. Only the variable Breed was not retained in the final model. The first month of lactation increased the odds of CM in heifers (Table 4, OR = 8.07, 95% CI 6.93–9.41). The final logistic regression model for heifers was well fitted according to residual deviance analysis (p-value > 0.05). The number of CM cases in the previous month of lactation (MAST1) showed the largest ORs. One CM case in the previous month increased the odds of a heifer having CM by more than 18 times (95% CI 15.70–21.32), and the OR for two CM cases was more than 10 (95% CI 9.00–15.33). SCC1 was positively associated with CM (OR = 2.34 per unit increase in SCC1). The rainy season showed increased odds compared to the dry season (OR = 1.21, 95% CI 1.12–1.31). The significant interaction between SCC1 and Season indicates that the effect of SCC1 was different in the rainy season compared to dry season ($OR = 2.34^{*}0.89^{x}$, where x is the number of units increase in SCC1).

Table 4
Summary of the final model of heifer-specific risk factors for clinical mastitis in the sample of 8 Brazilian dairy farms.

Variable	Value	*N*	% CM	Odds ratio	95% CI[a] for OR	*p* value
MIL[b]	1	4,888	10.62	8.07	6.93-9.41	<0.0001
	2 and above	44,698	6.96	1.00	Ref.[c]	Ref.
SCC1[d]	Continuous	–	–	2.34	2.24–2.44	<0.0001
MAST1[e]	0	47,555	5.95	1.00	Ref.	Ref.
	1	1,681	39.44	18.30	15.70–21.32	<0.0001
	2	350	30.43	11.75	9.00–15.33	<0.0001
MAST2[f]	0	35,493	5.34	1.00	Ref.	Ref.
	1	8,371	10.91	1.16	1.04–1.29	<0.05
	2 and more	5,722	14.37	1.19	1.04–1.37	<0.05
Season	Dry	25,309	6.15	1.00	Ref.	Ref.
	Rainy	24,277	8.55	1.21	1.12–1.31	<0.0001
Interaction	SCC1 × Season	–	–	0.89	0.85–0.93	<0.0001

[a] Confidence interval (CI).
[b] Months in lactation (MIL).
[c] Reference category (Ref.).
[d] Natural logarithm of SCC in previous month of lactation (SCC1).
[e] Number of CM cases accumulated in previous month (MAST1).
[f] Nnumber of all CM cases accumulated in all months prior to the previous month (MAST2).

3.2. CM in multiparous cows

The average annual incidence of new CM cases in multiparous cows per farm was approximately 31% per year, not including the repeated cases. Univariable analysis of cow-specific risk factors for CM in multiparous cows showed all factors were significant at $p < 0.25$. The final model included Parity, Breed, SCC1, SCC2, MAST1, MAST2 and Season (Table 5). The final logistic regression model for multiparous was well fitted according to residual deviance analysis (p-value > 0.05). Parity 3 and above had significantly lower odds compared to parity 2 (OR = 0.86; 95% CI 0.81–0.92). Each unit increase in SCC2, which represented the older history of SCC, increased the odds of CM by more than three times (OR = 3.40; 95% CI 3.19–3.63). An increase of one unit in the SCC2 represents an increase of 2.71 times in SCC. One CM case in the previous month of lactation (MAST1) increased the odds of having CM for multiparous cows by more than 20 times (OR = 20.02, 95% CI 18.4–21.75). *Bos taurus taurus* type also had a higher odds of having CM than *Bos taurus indicus* type (OR = 1.45, 95% CI 1.37–1.62). A significant interaction between SCC1 and Season was present showing decreased odds of CM with increasing SCC1 in the rainy season ($OR = 0.97 * 0.83^x$, where x is the number of units increase in SCC1).

3.3. Pathogens in CM cases

Coliforms (19%) were the most frequently detected pathogens followed by CNS (9%), other *Streptococci* (5%), *S. uberis* (5%) and *S. agalactiae* (4%). In 41% of the microbiological cultures no growth was observed. Contaminated cultures, which also included cultures with mixed pathogens, represented 5% of the total (Table 6).

In the multinomial analysis of coliforms, CNS, *S. uberis* and *S. agalactiae*, 580CM cases were included which represented 37% of the total number of microbiological culture results. Parity, Breed, SCC1, SCC2 and Season remained as significant factors in the final model (Table 7). Parity 2 and above showed reduced odds for having CM caused by CNS (OR = 0.19, 95% CI 0.11–0.32) compared to Parity 1 but higher odds for CM caused by *S. agalactiae* (OR = 1.56, 95% CI 1.25–1.96), using CM caused by coliforms as baseline. *Bos taurus taurus* type increased the odds of CM caused by CNS over five times (OR = 5.84, 95% CI 3.77–10.77) in comparison to *Bos taurus indicus* type. High SCC1 decreased the odds to be infected by SCN (OR = 0.48, 95% CI 0.40–0.58, for an increase of 1 unit of SCC1), whereas high SCC2 increased the odds of CNS (OR = 1.76, 95% CI 1.37–2.27) for an increase of 1 unit of SCC2). SCC2 also decreased the odds of CM caused by *Strep. agalactiae* (OR = 0.68, 95% CI 0.48–0.98). The rainy season decreased the odds of being infected by CNS in comparison to the odds of being infected in dry season (OR = 0.30, 95% CI 0.19–0.45).

4. Discussion

This study was performed using data from a non-randomly selected subpopulation of Brazilian dairy herds. Eight farms with available and good quality data on CM were chosen. These farms were considered to be good examples of specialized Brazilian dairy herds, given they have breeds, management technique and milk production per cow typical of specialized Brazilian dairy farms according to Zoccal et al. (2012). Because mastitis cases were defined by different milkers on eight different farms and the mastitis definition was subjective, we assumed that our study included variability in the definition of the mastitis cases among farms. To minimize the influence of herd particularities and seasonality on the model outcomes, farm and animal were included as random effects variables and the effect of the variation in the number of animals per year was also taken into account because the number of animals per year was considered as fixed effect. However, the influence of herd particularities and seasonality on the likelihood of cows having CM could be underestimated. Care was taken to reduce biases arising from the use of the non-random sample in this study.

Our results show that cow-specific factors influence the risk of having CM in Brazil, indicating that differences in susceptibility to CM exist among Brazilian cows, as identified in developed countries (e.g. Olde Riekerink et al., 2008; Steeneveld et al., 2008; Breen et al., 2009). At the same time, we found some differences in the factors that influence the occurrence of CM under Brazilian conditions. The mainly similarities and dissimilarities are discussed in this section.

The history of CM cases during a cowís lifetime is useful information for a mastitis control program. The number of CM cases in the previous month of lactation (MAST1) and the number of CM cases accumulated in the period prior to the previous month (MAST2) were risk factors for having CM. This finding is in line with the literature, although other studies found that MAST1 only increased the risk of CM by three to four times (e.g. Zadoks et al., 2001; Steeneveld et al., 2008; Breen et al., 2009). In our study, one mastitis case in the last month increased the chance of having CM by 18 times for heifers and 20 times for multiparous cows. This value was higher than expected and is most likely due to ineffective CM treatment on farms, which mask the symptoms but do not actually cure the mastitis, leading milkers to erroneously consider them as cured cases. Later, the symptoms become visible again, and milkers wrongly consider these as symptoms of a new infection. This leads to the overestimation of the number of CM cases per animal (Morant et al., 1988; Schroeder, 2012). The methodology applied in this study tried to control this misclassification, by defining a mastitis case to be a new case only if it occurred at least 14 days after the last CM case in the same animal. The high OR value for MAST1 suggests that this measure did not suffice to completely address the misclassification problem for this dataset.

Bos taurus indicus type (Zebu) play an important role in the composition of the Brazilian dairy herd, giving rusticity to cows and improving the effectiveness of milk production under tropical conditions and at low costs (Fonseca et al., 2009). Our results suggest Zebu probably have a lower chance of having mastitis than *Bos taurus taurus* type, probably due to their low genetic potential for milk yield (e.g. Wilson et al., 1997; Shem et al., 2002; Dego and Tareke, 2003). Within herds, high-yielding cows are generally more susceptible to mastitis than low-yielding cows (Dego and Tareke, 2003; Almaw et al., 2008).

Interactions between the SCC1 and Season were present. This is consistent with our expectation, because a pronounced association between season and udder health parameters is known (e.g. Green et al., 2006; Lievaart et al., 2007; Olde Riekerink, 2007).

Supporting this association, coliforms were involved in 18% of the CM cases for which a pathogen was identified in this study. Coliforms are so frequent because they occupy many habitats in the cow's environment, thus increasing the chances of contact and subsequent udder infection (Hogan and Smith, 2003). Repeated episodes of mastitis are expected because there are various pathogens that infect the mammary gland (Zadoks et al., 2011). Current routine control measures applied at milking time are ineffective. Reasons for this increase could be changes of housing (e.g., from stanchion barns to free stalls) or a direct result of reduced infection with staphylococci, streptococci, and the minor pathogens. These characteristics of this group, explains why mastitis due to environmental pathogens infection affects all dairy farms and why it is generally the major mastitis problem on well-managed dairy farms (Smith and Hogan, 1993; Hogan and Smith, 2003; Pyörälä, 2003; Zadoks et al., 2011).

After coliforms, CNS was the pathogen most frequently isolated in this study. CNS infections occasionally contribute to clinical cases

Table 5
Summary of the final model of multiparous cow-specific risk factors for clinical mastitis in the sample of 8 Brazilian dairy farms.

Variable	Value	*N*	% CM	Odds Ratio	95% CI[a] for OR	*p* value
Parity	2	42,813	7.39	1.00	Ref.[b]	Ref.
	3 and above	70,788	8.01	0.86	0.81–0.92	<0.0001
SCC1[c]	Continuous	–	–	0.97	0.92–1.01	0.14
SCC2[d]	Continuous	–	–	3.40	3.19–3.63	<0.0001
MAST1[e]	0	97,724	5.29	1.00	Ref.	Ref.
	1	14,599	39.44	20.02	18.4–21.75	<0.0001
	2	1,278	23.79	11.63	9.67	0.10
MAST2[f]	0	52,860	31.23	1.00	Ref.	Ref.
	1	20,107	10.59	1.20	1.09–1.32	<0.0001
	2	14,353	13.43	1.44	1.31–1.59	<0.0001
	3 and more	26,281	11.87	0.85	0.77–0.93	<0.0001
Breed[g]	0	32,027	6.49	1.45	1.37–1.62	<0.0001
	1	81,574	8.29	1.00	Ref.	Ref.
Season	Dry	59,394	5.38	1.00	Ref.	Ref.
	Rainy	54,207	10.41	20.77	12.5–34.54	<0.0001
Interaction	SCC1 × Season	–	–	0.83	0.8–0.86	<0.01

[a] Confidence interval (CI).
[b] Reference category (Ref.).
[c] Natural logarithm of SCC in previous month of lactation (SCC1).
[d] Geometric mean of all available natural logarithm of SCC of all months prior to the previous month (SCC2).
[e] Number of CM cases accumulated in previous month (MAST1).
[f] Number of all CM cases accumulated in all months prior to the previous month (MAST2).
[g] Breed: *Bos taurus taurus* type (0) and *Bos taurus indicus* type (1).

Table 6
Frequency of pathogens causing clinical mastitis determined by microbiological culture in milk samples of 8 Brazilian dairy herds.

Pathogens[a]	Number of cows			
	Heifers	Multiparous	Total	%
Coliforms	158	429	547	19
Coagulase-negative *Staphylococcus*	72	181	253	9
Streptococcus uberis	52	98	150	5
Streptococcus agalactiae	31	89	120	4
Corynebacterium bovis	32	66	98	3
Staphylococcus aureus	15	38	53	2
Bacillus sp	14	39	53	2
Streptococcus dysgalactiae	11	41	52	2
Yeast	4	36	40	1
Pseudomonas aeruginosa	10	26	36	1
Other *Streptococci*	42	116	158	5
Contaminated[b]	37	105	142	5
No growth	352	851	1.203	41
Total	830	2115	2905	100

[a] Only 23.30% of CM cases had microbiological culture results.
[b] Microbiological results with growth of more than one pathogen were also included in this category.

of mastitis in dairy herds, but CNS is rarely a major CM cause (Pyörälä et al., 2009). In pasture-based systems, such as in Brazil, a lower prevalence (16% in New Zealand) has been reported (Parker et al., 2007), which is consistent with the prevalence of 9% found in this study. CNS are pathogens commonly found on the teat skin and in the streak canal (e.g. Gillespie et al., 2009 Pyörälä et al., 2009). They are a common cause of contamination of milk samples and their isolation could also suggest poor sampling technique or poor teat end hygiene, or both (Pyörälä and Taponen, 2009).

A multinomial model was developed to analyze the influence of cow-specific risk factors on the chance of having CM caused by CNS, *Strep. uberis* and *Strep. agalactiae* infections in comparison to the chance of having CM caused by coliform infection. Our results showed that multiparous cows had lower probability of CNS infection in comparison to primiparous cows taking coliform infection as baseline. This result was consistent with our expectation, as older cows generally have a higher rate of clinical mastitis caused by coliforms compared to primiparous cows because of the length of time that they are exposed to environmental pathogens (Smith et al., 1985; Hogan and Smith, 2003).

Bos taurus taurus type had almost six times more chance of having a CM case caused by CNS compared with *Bos taurus indicus* type, taking coliform infection as baseline. More research on pathogen-specific susceptibility among different dairy cow breeds is needed to explain this finding.

We observed that high SCC in the recent history (SCC1) decreased the chance of CNS infection, whereas high SCC (SCC2) in the older history increased the chance of CNS infection, considering coliform infection as baseline. Although the results for recent SCC versus the older SCC history appear to be conflicting, both are justifiable results. CNS are common agents in subclinical mastitis and coliform are common agents in CM (e.g. Bradley, 2002; Hogan and Smith, 2003). High SCC in the previous month of lactation could indicate the presence of subclinical mastitis (e.g. Pyörälä, 2003). Because CNS infections tend to be subclinical and coliform infections tend to be clinical (e.g. Hogan and Smith, 2003; Piessens et al., 2011; De Vliegher et al., 2012), the chance of CM caused by coliform infection will be greater. At the same time, it has been found that even some well-managed herds, which maintain low SCC, find it difficult to control coliform

Table 7
Summary of the final multinomial logistic regression model of cow-specific risk factors for clinical mastitis (CM) caused by infection of coagulase-negative *Staphylococcus*. Coliforms. *Streptococcus agalactiae* and *Streptococcus uberis* using CM infection by Coliforms as baseline and data of 8 Brazilian dairy herds.

Variable	Value	Odds Ratio	95% CI[a] for OR	*p* value
Coagulase negative *Staphylococcus* (baseline Coliforms)				
Parity	1	1.00	Ref.[b]	Ref.
	2 and above	0.19	0.11–0.32	<0.0001
Breed[c]	0	1.00	Ref.	Ref.
	1	5.84	3.77–10.77	<0.0001
SCC1[d]	Continuous	0.48	0.40–0.58	<0.0001
SCC2[e]	Continuous	1.76	1.37–2.27	<0.0001
Season	Dry	1.00	Ref.	Ref.
	Rainy	0.30	0.19–0.45	<0.0001
Streptococcus uberis(baseline Coliforms)				
Parity	1	1.00	Ref.	Ref.
	2 and above	1.30	0.66–2.54	0.49
Breed	0	1.00	Ref.	Ref.
	1	0.81	0.25–1.88	0.99
SCC1	Continuous	1.09	0.73–1.52	0.28
SCC2	Continuous	0.70	0.29–1.25	0.11
Season	Dry	1.00	Ref.	Ref.
	Rainy	1.19	0.71–1.99	0.93
Streptococcus agalactiae(baseline Coliforms)				
Parity	1	1.00	Ref.	Ref.
	2 and above	1.56	1.25–1.96	<0.0001
Breed	0	1.00	Ref.	Ref.
	1	0.71	0.33–1.50	0.42
SCC1	Continuous	1.23	0.84–1.68	0.09
SCC2	Continuous	0.68	0.48–0.98	<0.05
Season	Dry	1.00	Ref.	Ref.
	Rainy	0.92	0.46–1.62	0.95

[a] Confidence interval (CI).
[b] Reference category (Ref.).
[c] Breed: *Bos taurus indicus* type (1) and *Bos taurus taurus type* (0).
[d] Natural logarithm of SCC in previous month of lactation (SCC1).
[e] Geometric mean of all available natural logarithm of SCC of all months prior to the previous month (SCC2).

mastitis, and may even experience a higher incidence of disease (e.g. Pyörälä, 2003). Given this, an older history of high SCC may affect the chance of CNS infection more than the chance of coliform infection.

The rainy season was negatively associated with having CM from a CNS infection compared to dry season, taking coliform infection as baseline. This negative association may be due to coliform levels in the environment increase during warm and wet months. Additionally, it is possible that increases in clinical coliform mastitis are caused by heat stress affecting the susceptibility of the mammary host defenses to Gram-negative bacteria, such as coliform (Hogan and Smith, 2003).

Multiparous cows had a higher odds of having *S. agalactiae* infections than primiparous cows, taking coliform infection as baseline. *S. agalactiae* is a highly contagious obligate parasite of the bovine mammary gland. The virulence of this pathogen is higher than of coliforms (e.g. Keefe, 1997; Krishnaveni et al., 2014). Breen et al. (2009) showed that *S. agalactiae* had a higher odds to infect the cows compared to coliforms. Additionally, the coliform infection rate is higher during the dry period, when there is no milk production, than during the lactation period (Smith et al., 1985). The odds is even greater in multiparous cows because they are older and have a higher probability of having had contact with pathogens than primiparous cows. Given these observations, this result is in line with our expectations.

We also found that a high accumulated SCC reduced the odds of a CM infection by *S. Agalactiae*, considering coliform infection as baseline. Given the other findings of this study, where a high SCC was a risk factor for CM and coliforms were almost five times more frequent than *S. agalactiae*, it is likely that this result just reflects the higher likelihood of coliform infection compared to *S. agalactiae* infection in the herds analyzed. A high accumulated SCC cannot be considered as factor that decrease the probability of CM infection by *S. Agalactiae* based only on this result, given that *S. agalactiae* is one of the most common pathogens in high SCC dairy herds, as shown by different authors (e.g. Erskine et al., 1988; Barkema et al., 1999; Schukken et al. (2009)).

In summary, our results suggest that CM control programs should focus on the control of environmental pathogens, mainly during the rainy season. This is the most promising way to reduce the occurrence of CM in Brazilian dairy cattle based on the study outcomes.

5. Conclusions

Parity, months in lactation, breed, season, SCC and especially CM history were important cow-specific factors influencing the likelihood of CM under Brazilian conditions. The presence of CM cases in the most recent CM history substantially increased the odds of having CM, illustrating the importance of registering CM information in order to design better control strategies. Among the pathogens analyzed, the influence of cow-specific risk factors was different for CNS and for *S. agalactiae* in comparison to coliforms. The environment was the main source of CM pathogens, with greater involvement of coliforms.

Acknowledgments

The authors thank Coordination for the Improvement of Higher Education Personnel (CAPES) and National Counsel of

Technological and Scientific Development (CNPq) (140634/2012-6), and Capes PDSE (2278-14-6) for the financial support and scholarship for the first author. The authors also thank the University of Utrecht, the Netherlands, for the facilities and cooperation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2015.08.001.

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