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

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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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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