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An investigation of risk factors for two successive cases of clinical mastitis in the same lactation

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ABSTRACT

The objective of this study was to identify risk factors for the occurrence of two successive cases of clinical mastitis (**CM**). Two farms were visited monthly during 10 months. Milk samples were collected from all cases of CM that occurred during the study. Cows were observed prospectively from the beginning of the study and a case cow was defined when she experienced the second case of CM within the same lactation. For each case cow, 3 control cows matched by days in milk (**DIM**) were randomly selected from the cohort of cows who did not experience CM. On each visit day, a series of udder and teat characteristics were recorded during milking time: teat-end hyperkeratosis scoring, milking ease scoring, teat length and diameter, teat-to-ground distance, and udder position in relation to the hock. A total of 113 case cows and 324 control cows were used for analyses. The median time to occurrence of the first case of CM was 84 DIM and the median interval between the first and second cases of CM was 39 days. Of all second cases, 49.6% (N=55) occurred in the same mammary gland. Of these 55 cases, 29.1% had identical milk culture results from both first and second cases. Most cases of CM were caused by coliforms and environmental streptococci. Teat-to-ground distance, teat-end hyperkeratosis, udder position in relation to the hock, milking ease, parity, and milk production at the first test of lactation were individually associated with the occurrence of two successive cases of CM. Of all explanatory variables, 3 remained in the final multivariable model. The odds of two successive cases of CM were 3.7 times greater for cows who were “very easy to milk”, as compared with cows who were “difficult to milk”. Cows who had their udders below the hock, and those of parity > 2 were 3.6 and 2.5 times more likely to experience two successive cases of CM, as compared with cows whose udder was positioned above the hock, and cows of parity 1, respectively. Findings of this study highlight the importance of teat and udder characteristics as risk factors for two successive cases of CM. Further investigations are needed to elucidate the role of the teat canal in preventing mastitis for modern cows that have been selected for increased milk production, shorter teats, and greater milk flow rates.

1. Introduction

Control of clinical mastitis (**CM**) is increasingly challenging on farms that produce high-quality milk worldwide. Results of studies conducted in developed dairy regions and including large populations of herds indicate that 20.1–40% of dairy cows experience CM every year (Barnouin et al., 2005; Bradley et al., 2007; Olde Riekerink et al., 2008). The cost of a single case has been estimated between €$ 210 and €$ 287 (Halasa et al., 2007; Huijps et al., 2008), resulting in great economic losses to producers.

Changes in mastitis etiology, genetic selection of cows, and management practices are factors that have contributed to increasing the burden of CM on modern dairy herds. Environmental pathogens such as coliforms, which are primarily associated with CM, have become more prevalent due to successful control and eradication of contagious pathogens (Lago et al., 2011; Oliveira and Ruegg, 2014). Cows have also been intensively selected to increase milk production and milking speed (i.e., milk flow rate), with the former being consistently correlated with increased susceptibility to CM (Rupp and Boichard, 2003). Moreover, maintenance of teat health has been challenging on large dairy operations due to increasing exposure to management practices used towards maximizing milk production (e.g., 3-times-a-day milking) and minimizing milking time (e.g., milking machines settings).

In this context, identification of risk factors is needed to prevent and manage CM at the farm level. Several cow factors have been

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associated with the occurrence of CM, among which are parity (Breen et al., 2009; Hertl et al., 2014), milk production (Hertl et al., 2014), stage of lactation (Olde Riekerink et al., 2008; Breen et al., 2009), somatic cell count (**SCC**) previous to CM (Green et al., 2004; Steeneveld et al., 2008), breed (Barkema et al., 1999), and teat pathologies and anatomical characteristics (Grindal and Hillerton, 1991; Neijenhuis et al., 2001; Breen et al., 2009).

It has also been consistently demonstrated that cows that experience CM are more likely to develop further cases in both current and subsequent lactations (Bar et al., 2007; Steeneveld et al., 2008; Pantoja et al., 2009; Hertl et al., 2014). Cows who experience repeated cases of CM are a relevant issue for dairy producers because there is frustration due to repeated treatment failures and cumulative economic losses throughout lactation. Pinzón-Sánchez and Ruegg (2011) reported that the overall bacteriological cure rate after intramammary treatment with ceftiofur was less (51.9%) for cows who experienced previous cases of CM, than that observed for cows who did not experience any previous case in the same lactation (86.5%).

Milk production losses accumulate with the number of additional cases of CM in the same lactation. As compared to the projected lactational milk yield of healthy cows, average reductions were of 253, 238 and 216 kg within 60 days after cows developed the first, second and third case of CM in lactation, respectively (Bar et al., 2007). The pattern of milk loss also depends on parity, causative agent, and similarity of the pathogens causing subsequent episodes of CM (Hertl et al., 2014). Moreover, cows who experience repeated cases of CM are at greater risk of dying or being prematurely culled from the herd (Bar et al., 2008).

It is well defined that chronic intramammary infections, which can alternate between subclinical and clinical states, and treatment failures are causes of repeated episodes of CM (Bradley and Green, 2001; Hillerton and Kliem, 2002). Nonetheless, on modern farms, in which the distribution of pathogens is mostly comprised of environmental organisms, most repeated cases of CM are caused by different species of pathogens, or different strains of the same species (Abureema et al., 2014), suggesting that cow factors unrelated to pathogens play a major role in increasing the susceptibility to repeated episodes of CM.

The objective of this study was to identify risk factors for the occurrence of two successive cases of CM in the same lactation, with a focus on udder and teat physical characteristics that could be managed at the farm level, or by means of genetic selection.

2. Methods

This research was approved by the UNESP´s Ethics Committee for Animal Use (protocol 156/2013).

2.1. Farm selection and herd characteristics

A convenience sample of 2 dairy farms was selected for the study. Herds were eligible to participate if located in Sao Paulo State, Brazil, were composed of >300 Holstein lactating cows, used computerized animal records (health and production data), adopted systematic measures to control major contagious mastitis pathogens (*Streptococcus agalactiae* and *Staphylococcus aureus*), and were willing to comply with the study protocol.

Herds A and B were composed of 400 and 1700 lactating cows who produced an average of 35 and 41 kg of milk/day, with bulk tank milk SCC of 346,000 and 366,000 cells/mL, respectively. Cows of herd A were housed in a free-stall barn bedded with rubber mattresses and sawdust, and milked twice a day in a rotary parlor. Cows of herd B were housed in a cross-ventilated free-stall barn bedded with sand and milked 3 times a day in a rotary parlor. Both farms adopted mastitis control programs based on the NMC´s 10-point mastitis control plan (National Mastitis Council) and used complete milking routines consisting of visual examination of foremilk, pre-dipping with chlorine (herd A) and iodine (herd B) solutions, drying of teats with individual cloth (herd A) and paper (herd B) towels, milking of cows followed by automatic cluster detachment, and use of post-dipping with iodine solutions.

Both farms used standardized treatment protocols for CM. Mild and moderate cases were treated primarily with 3–5 intramammary infusions of ceftiofur (Spectramast, Zoetis, NJ, USA) or cefquinome (Cobactan, MSD Animal Health, NJ, USA). Severe cases were treated with the protocols aforementioned, in addition to supportive and systemic antimicrobial therapy.

2.2. Study design and data collection procedures

We conducted a case-control study with incidence density sampling (Dohoo et al., 2010). Farms were initially visited to explain the study protocol and obtain informed consent. Milking technicians and herd managers were trained by study personnel for standardized detection of CM and collection of aseptic milk samples.

Farms were visited monthly for collection of data and frozen milk samples. Farm A was visited between July 2013 and April 2014, and Farm B was visited between June 2014 and March 2015. Clinical mastitis was detected at every milking by milking technicians, by visual examination of foremilk. Milking technicians were asked to collect aseptic milk samples (15 mL) from all cases of CM that occurred during the study, before administration of intramammary treatment, or at detection for cases that were not treated. Samples were frozen on the farms and collected monthly by study personnel. Cows were treated according to each farm´s standardized protocol and information pertaining to each case (number of quarters affected and treatment protocol) was recorded. Production data, such as monthly dairy herd improvement association (**DHI**) milk production and SCC, parity, and days in milk (**DIM**) were obtained from the farms´ management software.

2.3. Definition and selection of cases and controls

Clinical mastitis was defined as the presence of milk visual abnormalities (such as flakes, pus, and changes in color), which could be accompanied or not by alterations in the mammary gland (e.g., swelling, redness and pain) and in the systemic state of the cow (e.g., fever and dehydration).

Cows were eligible to be cases or controls if they were in lactation and included in the DHI monthly testing program. Cows were observed prospectively from the beginning of the study and a case cow was defined when she experienced the second case of CM within the same lactation, regardless of the quarter affected. Only cases that occurred after 14 days from a previous case were considered new (Bar et al., 2007; Hertl et al., 2014). For each case cow, 3 control cows matched by days in milk (± 15 days) were randomly selected using PROC SURVEY SELECT (SAS Institute, Cary, NC, USA) from the cohort of cows who did not experience any case of CM.

A list including cases and controls was produced monthly, before each visit day, to identify cows for field measurements. Control cows were eligible to become cases if they experienced repeated cases of CM afterwards. Cows could have been selected as controls more than once during the study, as part of the random selection procedure (Dohoo et al., 2010).

2.4. Field measurements

On each visit day, a series of udder and teat characteristics were recorded during milking time. Investigators were masked to the case-control status of each animal. After milking of each selected animal, the following measurements were performed on udders and 2 contralateral teats (e.g., right front and left rear teats of a cow, and left front and right rear teats of the next cow): 1) teat-end hyperkeratosis scoring: 4-