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The association between subclinical mastitis around calving and reproductive performance in grazing dairy cows

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A R T I C L E I N F O

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A B S T R A C T

The objective of this study was to evaluate the effect of subclinical mastitis (SCM) on calving-to-first-service interval (CFS), calving-to-conception interval (CC), and on the number of services per conception (S/C) in grazing Holstein and Normande cows. Primiparous (n = 43) and multiparous (n = 165) cows were selected from five dairy herds. Two composite milk samples were aseptically collected from each cow at drying-off, and then every week during the first postpartum month. One sample was used for somatic cell count (SCC), and the other one for bacteriological analysis. Cows were followed up to 300 d after calving. Non-parametric and parametric survival models, and negative binomial regression were used to assess the association between SCM, evaluated by SCC and milk culture, and reproductive indices. *Staphylococcus aureus*, CNS, and *Streptococcus uberis* were the most frequent isolated pathogens. Subclinical mastitis in the first month of lactation was not associated with CFS; however, the CC interval was longer in cows with SCM compared to healthy cows, the former also had a higher number of S/C.

1. Introduction

Bovine mastitis is a costly disease that affects the profitability of the milk industry due to the reduction of milk yield and the high amount of discarded milk, early culling of cows, and treatment costs. The cost of the udder health programs has been estimated to be, at least, one third of the total money invested for preventing diseases in dairy herds (Fourichon et al., 2001; Bar et al., 2008) The reproductive performance of the herd has also an impact on the economic results of the dairy business, as low reproductive efficiency may cause a decrease in milk production, and in the number of calves born per year (Maizon et al., 2004).

Fertility in dairy herds has declined in the past five decades while milk yield has increased (Walsh et al., 2011). Nevertheless, how much variation in cow fertility explained by high milk yield remains unclear, as fertility is affected by other factors than milk yield (e.g. heat detection, nutrition, environment, stress, and cow's genetics and health status) (Dohoo et al., 2001; LeBlanc, 2010). In recent years, studies on the physiology of reproduction in different species have looked for different explanations on how physiological, immunological and pathological processes have an impact on fertility (Pate et al., 2010; Bromfield and Sheldon, 2011). For example in cows experiencing intramammary infections (IMI), inflammatory and immune responses are activated outside the reproductive organs that can lead to embryonic death (Soto et al., 2003).

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Recent studies found that cows with clinical mastitis had a significant decrease in their first service conception rate, and a reduced probability of pregnancy compared to healthy cows (Fuenzalida et al., 2015). Likewise, cows with clinical IMI have a higher number of services per conception (S/C), and longer interval from calving-to-first-service (CFS) or calving-to-conception (CC) than that of healthy cows. It has been reported that S/C were higher in cows with clinical cases after their first service than in cows with clinical mastitis before the first service (Gunay and Gunay, 2008). Some other studies have revealed the association between clinical mastitis and failures to become pregnant after a service (Hertl et al., 2010; Hertl et al., 2014).

Meta-analytic studies suggested that reproductive pathologies occurring around calving, such as retained placenta and ovarian cysts, might negatively affect the reproductive performance (i.e. increasing of intervals from calving-to-first-service; CFS, and CC); however, clinical mastitis did not show a significant effect on the reproductive performance (Fourichon et al., 2000). Nevertheless, an increased production of interleukins and mediators of inflammation (i.e. prostaglandins and cortisol) may mediate the effect of mastitis, either subclinical or clinical, on the reproductive performance. These compounds may have a negative effect on the development and quality of oocytes and corpus luteum, affecting the length of the estrous cycle in cows (Risco et al., 1999; Schrick et al., 2001).

On the other hand, there are studies that found associations between subclinical mastitis (SCM), as measured by increased somatic cell counts (SCC) in milk, and impaired reproductive performance (Hudson et al., 2012; Pinedo et al., 2009). Studies conducted in temperate zones found a reduction of 44% in the risk of pregnancy when cow SCCs increased above 90,000 cells/mL before breeding (Pinedo et al., 2009). Dairy cows in grazing systems in Argentina that had SCM (i.e. SCC > 200,000 cells/mL) were 2.56 times more likely to have more than two services during the breeding season compared to healthy cows (Gómez-Cifuentes et al., 2014).

Colombia is located in the northwest corner (4° 35′ N, 74° 4′ W) of South America near to the equator. The Colombian climate is characterized as tropical and isothermal, which causes a significant regional variation in precipitation and temperature (CENICAFE, 2016) in the pasture-based dairy systems. These systems tend to have gram-positive bacteria dominating effects on udder health (Ramírez et al., 2014; Reyes et al., 2015; Villa-Arcila et al., 2017), which contrasts to more temperate regions of the world (Bradley, 2002; Shum et al., 2009). These latter regions have equivocal reports on the effect of specific mastitis pathogens on the reproductive performance in dairy cows (Fourichon et al., 2000; Fuenzalida et al., 2015).

In Colombian herds *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium pyogenes* are highly prevalent (Ramírez et al., 2014; Villa-Arcila et al., 2017) but there appear to be no specific studies conducted to evaluate what is the effect of these infections on the reproductive performance of dairy cows grazing in tropical regions. Therefore, the main objective of this study was to evaluate the hypothesis that SCM occurring in the first month after calving negatively affects the intervals from CFS and CC, and the S/C in dairy cows grazing in tropical systems.

2. Materials and methods

A longitudinal study was designed, and conducted between June 2013 and September 2014. Five dairy herds located in Manizales and Villamaria, Colombia were enrolled. Herds were located at 5° 1′ N and between 75° 22′ and 75° 27′ W. Herds were selected due to the willingness of the owner to participate in the study, and if the farmers had: permanent veterinary advisory service, records from each cow of the herd, and a health management plan to prevent infectious reproductive disorders (e.g. brucellosis, infectious bovine rhinotracheitis, and bovine viral diarrhea), and other infectious diseases, such as leptospirosis, and foot and mouth disease. These dairy herds were more progressive compared to the average herds in the region. The average herd size in our study was lower than the mean of 68 lactating cows observed for herds in Manizales and Villamaria, Colombia. The mean number of lactating cows in our study herds was 41.6 (ranging from 24 to 80).

The sample size for the study was calculated under the assumption of a high incidence risk of IMI around calving in Colombian dairy herds, which can be as high as 25%, according to unpublished data from DHI records and previous information from our Laboratory of Milk Quality at Universidad de Caldas. Therefore, the incidence risk of an IMI in cows around calving was set at 0.20 (exposed) to calculate the required sample size. We expected to have a relative hazard of 0.60 (i.e. hazard in exposed cows/hazard in unexposed cows), with a confidence of 95% and a study power of 0.80. Consequently, 188 events in total were required for our study (Schoenfeld, 1983).

Cows (n = 208; primiparous = 43, multiparous = 165) were selected by convenience according to their expected calving date. Signs of clinical mastitis at drying-off were not observed, and cows had no blind quarters. Cows were assigned to one of two groups according to their breed: Holstein, n = 151 or Normande, n = 57. A total of 165 cows were multiparous and 43 were primiparous. The general characteristics of the herds were described elsewhere (Villa-Arcila et al., 2017). Briefly, herds were managed under rotational grazing systems, and cows received supplementation with concentrates according to milk yield. The predominant pasture was a mixture of Kikuyu grass (*Pennisetum clandestinum*), Orchardgrass (*Dactylis glomerata* L.), and Yorkshire fog grass (*Holcus lanatus*). Concentrates used were commercial mixes of cereals, containing 14% to 16% of crude protein, and approximately 2.9 Mcal of metabolizable energy/kg of dry matter. Concentrates were fed starting three wk before calving (2 kg/cow/d), and at a rate of 1 kg per 4 kg of milk yield after calving. Mineral supplements and water were available *ad libitum*.

2.1. Sampling

Composite milk samples show relatively high sensitivity for the diagnosis of major mastitis pathogens, while the sensitivity for *Strep. uberis* and minor pathogens is moderate. Nevertheless, increasing the number of affected quarters improves the sensitivity of the analysis. The specificity is high no matter what pathogen is involved (Reyher and Dohoo, 2011). Therefore, two samples of