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## Herd- and cow-level risk factors associated with subclinical mastitis in dairy farms from the High Plains of the northern Antioquia, Colombia

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### ABSTRACT

Mastitis is the main disease entity affecting dairy farms in the Colombian High Plains of northern Antioquia, Colombia. However, no previous epidemiologic studies have determined the characteristics that increase the risk of infection in this region, where manual milking is still the prevailing system of milking. A 24-mo longitudinal study was designed to identify the predominant mastitis pathogens and important herd- and cow-level risk factors. Monthly visits were made to 37 commercial dairy farms to collect herd- and cow-level data and milk samples. Herd size varied from 6 to 136 cows (mean 37.0, median 29). Herd-level factors included type of milking system (manual or mechanical) and a range of management practices recommended by the National Mastitis Council (Madison, WI) to prevent mastitis. Individual cow-level risk factors included parity, stage of lactation, breed, udder hygiene, and lameness. A logistic regression analysis was used to investigate associations between herd- and cow-level risk factors with the presence of subclinical mastitis and infection caused by *Streptococcus agalactiae* at the quarter level. A quarter was considered to have subclinical mastitis if it had a positive California Mastitis Test and was subsequently confirmed to have a somatic cell count of  $\geq 200,000$  cells/mL. Any cow with one or more quarters with subclinical mastitis was considered to have subclinical mastitis at the cow level. Using 17,622 cow observations, the mean prevalence of subclinical mastitis at the cow level was 37.2% (95% confidence interval: 31.2, 43.3) for the first month and did not substantially change throughout the study. The predominant microorganisms isolated from quarters meeting the subclinical mastitis definition were contagious pathogens, including *Strep. agalactiae* (34.4%), *Corynebacterium* spp. (13.2%), and *Staphylococcus aureus*

(8.0%). Significant variables associated with subclinical mastitis risk at the quarter level included being a purebred Holstein cow, higher parity, and increased months in milk. Variables that were protective for mastitis risk included being a crossbreed cow and adequate premilking udder hygiene. Significant variables associated with *Strep. agalactiae* infection were higher parity, increased months in milk, and manual milking. Variables that were protective were postmilking teat dipping and adequate cleaning of the udder. The results highlight the importance of hygiene practices in contagious mastitis control in manually milked herds.

**Key words:** mastitis prevalence, manual milking, dairy cow

### INTRODUCTION

Mastitis is globally considered to be the most costly infectious disease in the dairy industry (Fetrow et al., 1991). In the United States alone, calculated losses were approximately \$2 billion per year during the 1990s (Harmon, 1994) and for countries such as Germany, recent estimates attribute losses of €200/cow per year (Kramer et al., 2009). Economic data for Colombia are not available. However, studies using the California Mastitis Test (CMT) and SCC as tools to diagnose subclinical mastitis (SM) have reported prevalence values that range from 19.9 to 51.3% (Calderon and Rodríguez, 2008; Pinzon et al., 2009; Trujillo et al., 2011). Other authors have recently reported that contagious mastitis caused by *Streptococcus agalactiae* was associated with a 70% increase in herd SCC and a near doubling of raw milk bacteria counts in Colombia (Keefe et al., 2011).

Risk factors for SM, including the importance of postmilking teat disinfection, have been established for many years (Neave et al., 1969). Prevention of the spread of pathogens from cow to cow implies that the teats must be kept free of pathogens. To this end, methods have been investigated, including the use of disinfectants, paper towels, or individual wash cloths; the

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wearing of rubber gloves by the milker; and the cleaning and disinfecting of the teat cup clusters before each cow is milked, together with postmilking disinfectant teat dips aimed at destroying any pathogens remaining on the teats after milking (Neave et al., 1969). One of the techniques used to monitor the level or occurrence of SM in herds or individual cows or quarters is to determine the SCC (Dohoo and Meek, 1982). Most practices having consistent associations with SCC were related to milking procedures: using automatic take-offs, using postmilking teat dipping (**PMTD**), milking problem cows last, yearly inspection of the milking system, and use of a technique to keep cows standing following milking; all were consistently associated with lower herd SCC (Dufour et al., 2011). Because the conditions leading to a high prevalence of mastitis are numerous, mastitis prevention programs should ideally be preceded by an assessment of the risk factors most relevant to the local industry. In the northern plains of Antioquia, Colombia, approximately 90% of producers still use manual milking, which is carried out directly on the pasture (Ramírez et al., 2009). Locally reported studies and anecdotal evidence suggest that bovine mastitis is undoubtedly the main disease entity affecting dairy cows in this area (Ramírez et al., 2001; Rodríguez et al., 2002). However, rigorous studies that identify the primary risk factors associated with subclinical mastitis are lacking in Colombia. In various cross-sectional studies conducted in Antioquia and the Bogota savannah, a positive association was observed between SM (as determined by positive CMT), parity, and mechanical milking (Ramírez et al., 2001). By contrast, no relationship was found with days in lactation (Ramírez et al., 2001). The main bacterial species isolated from mastitic Colombian cows have been *Strep. agalactiae* and *Staphylococcus aureus*, which accounted for 35 to 45% and 14 to 33% of infections, respectively (Rodríguez et al., 2002; Rodríguez, 2006). Because manual milking is the prevailing milking system in many parts of Colombia, we were interested in establishing the important risk factors associated with the prevalence of SM and infection by contagious pathogens in these herds.

The 2 objectives of this SM study were (1) to obtain overall and bacterial pathogen-specific prevalence estimates, and (2) to evaluate the major risk factors for SM in typical dairy farms of the High Plains region of Colombia.

## MATERIALS AND METHODS

### Ethical Considerations

This research was approved by the Ethics Committee for Animal Experimentation of the University of

Antioquia, Colombia (Act number 48, from December 12, 2008).

### Herd Selection

A convenience sample of 37 herds were selected from 3,049 registered dairy farms in 6 municipalities that make up the main dairy-specialized area of the High Plains of northern Antioquia, Colombia (Entrerrios, Belmira, Santa Rosa de Osos, San Jose de la Montaña, San Pedro de los Milagros, and Donmatias). Based on the inclusion criteria described below, 120 typical herds from the 6 municipalities were invited to participate, 99 accepted, 21 declined, and 37 were selected for sampling. The final 37 herds selected were those that best met the characteristics defined below. The number of herds selected in each municipality was proportional to the size of that area's dairy industry: Belmira 9% (3 herds), Donmatias 11% (3 herds), Entrerrios 19% (6 herds), San Jose 5% (2 herds), San Pedro 24% (8 herds), and Santa Rosa 32% (10 herds). Within each municipality, herds were selected based on the following typical management conditions in the region, including 2 milkings a day, the use of pasture as the main source of feed, giving concentrate to the cows as a supplement at milking time, AI, breeds mainly composed of specialized dairy cows, on-farm cooling tanks, and proper cow identification. Other aspects taken into account to select the herds were easy road access and whether the owner was willing to collaborate in collection of samples. When it was necessary to replace one of the herds, investigators selected another herd from the list of eligible herds in that region that met the inclusion criteria. All cows in each herd selected were included in the study. Based on previous work that reported a cow-level prevalence estimate for SM of 34% (Ramírez et al., 2001) and a desired precision of  $\pm 5\%$ , the number of animals required for a single-point-in-time prevalence estimate was 324 cows (SPSS Statistics, version 21.0; IBM Corp., Armonk, NY). To adjust for the reality that true random sampling could not be conducted because of industry logistics and the fact that the level of herd clustering was not accounted for in this sample size calculation, the total number of cows sampled at any sampling time was approximately 700 cows. All the milking cows in each herd were sampled each time. The study started with 32 herds that met the inclusion criteria. However, 5 had to be replaced part-way through the study so data were obtained from 37 different herds.

### Visit Protocol

All farms were visited once per month for a period of 2 yr (January 2009 to December 2010) to collect

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