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Short communication: Molecular epidemiology of *Streptococcus agalactiae* differs between countries

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ABSTRACT

Group B *Streptococcus* or *Streptococcus agalactiae* continue to be challenging for milk quality programs in countries with emerging dairy industries, such as Colombia, where high prevalence has been reported. Molecular typing of isolates is needed to understand the variability and epidemiology of this pathogen and to develop effective control and eradication programs. We characterized the molecular profile of *Strep. agalactiae* isolated from cows with subclinical mastitis in 21 Colombian dairy herds and measured diversity within and between herds using multilocus sequence typing. Isolates belonged to sequence type 248 [clonal complex (CC) 103; n = 30], ST1 (CC1; n = 6) or ST22 (CC22; n = 4)], whereas members of CC67/61, the dominant type in North America, were not detected. Presence of multiple clonally unrelated sequence type within a herd was common, which contrasts with the situation in European countries and suggests introduction from multiple sources. Our results demonstrate that conclusions from molecular epidemiological studies in 1 region cannot necessarily be extrapolated to other regions, and no single bovine-adapted CC of *Strep. agalactiae* exists in Colombia. Improvements in internal and external biosecurity will be needed to reduce *Strep. agalactiae* prevalence in Colombian dairy herds.

Key words: subclinical mastitis, *Streptococcus agalactiae*, molecular epidemiology

Short Communication

Streptococcus agalactiae or Group B *Streptococcus* is a common cause of bovine subclinical mastitis in countries with emerging dairy industries and is a re-emerging pathogen in European countries with long-standing mastitis control programs (Katholm et al., 2012; Keefe, 2012). Group B *Streptococcus* is also an important pathogen in people. It is the major cause of infectious disease in neonates in western countries and it is recognized as emerging pathogen in adults worldwide. People can also be asymptomatic carriers of the organism (Brochet et al., 2006; Lyhs et al., 2016). *Streptococcus agalactiae* is a very common pathogen of dairy cattle in several regions of Colombia, with prevalence estimates of 11 to 60% at the herd level (Keefe et al., 2011) and 35% at the cow level (Ramírez et al., 2014).

Molecular methods have been used to gain insight into the pathophysiology and epidemiology of *Strep. agalactiae* in dairy herds, whereby different methods serve different purposes (Zadoks et al., 2011). Comparative methods, such as pulsed-field gel-electrophoresis and random amplified polymorphic DNA (RAPD) typing, have been used for within-herd studies over short spatiotemporal scales because of their relatively high discriminatory power, whereas multilocus sequence typing (MLST) is more suited for studies at a large spatiotemporal scale, such as comparison of populations across herds or countries, because it is a universal typing system based on slowly evolving housekeeping genes (Mahmmod et al., 2015; Jørgensen et al., 2016; Lyhs et al., 2016). Whole-genome sequencing has primarily been used to study host adaptation of *Strep. agalactiae*

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(Richards et al., 2011; Richards et al., 2013; Almeida et al., 2016), and may replace other methods as a tool for population-level studies now that next-generation sequencing is making it increasingly rapid and affordable. When MLST was first applied to bovine isolates, it was suggested that a single bovine-adapted clonal complex (CC), CC67, was responsible for the majority of bovine mastitis (Bisharat et al., 2004). All isolates in that study originated from the United Kingdom, and CC67 is still the most common type of *Strep. agalactiae* in Scotland (R. Zadoks, unpublished data). Different types of *Strep. agalactiae* dominate in cattle in studies from other countries (e.g., CC1 in northern Europe or CC103 in China; Zadoks et al., 2011; Yang et al., 2013; Lyhs et al., 2016). Little is known about the dominant CC of *Strep. agalactiae* in South America in general, or about its molecular epidemiology in Colombia in particular. The aim of the current study was to investigate the sequence type (ST) and CC present in a collection of *Strep. agalactiae* isolates from Colombian dairy herds to identify important strains, potential sources, and routes of transmission.

Isolates for this research were part of Ramírez et al. (2014) study (January 2009 to August 2011) in the northern region of Antioquia, Colombia. Pre- and post-milking teat disinfection were used in most study herds (90.4%), and hand milking was twice as common as machine milking. Within-herd heterogeneity was initially assessed using RAPD typing was conducted on isolates from 100 milk samples obtained from 66 cows with subclinical mastitis originating from 23 herds, whereby subclinical mastitis was defined as a cow-level SCC above 200,000 cells/mL in the absence of visible abnormalities of the milk or udder (Reyes Velez, 2016). For DNA extraction, individual colonies of *Strep. agalactiae* were collected from culture plates and suspended in 1.5 mL of tripticase soy broth with overnight incubation at 37°C. Genomic DNA was extracted with the DNeasy Blood & Tissue Kit following the manufacturer's procedure (Qiagen, Hilden, Germany), resuspended in 100 mL of TE buffer (10 mM Tris, 1 mM EDTA) and stored in Eppendorf tubes. The DNA was sent to the Atlantic Veterinary College. Quantity and quality of DNA were assessed using 1 µL of the sample and measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific Inc., Wilmington, DE); RAPD typing was performed to determine within-herd heterogeneity (data not shown; Reyes Velez, 2016). Based on RAPD typing, 1 or 2 types per herd were identified. (Reyes Velez, 2016). Using 1 isolate per RAPD type per herd, 40 isolates from 35 cows in 21 herds were selected for MLST. Two isolates from 2 different herds were not considered for further MLST analysis due to low DNA quality. The MLST was implemented using the high-throughput

HiMLST method at Streeklab Haarlem (Haarlem, the Netherlands; Boers et al., 2012). Allelic profiles and ST were assigned to each isolate through comparison with the PubMLST database for *Strep. agalactiae* (<http://pubmlst.org/sagalactiae>) and grouped using eBURST (based upon related sequence types) version 3 software (<http://eburst.mlst.net/>; Feil et al., 2004). To avoid amalgamation of previously separate CC into a single CC, CC were named using modified nomenclature as also implemented in other studies (Lopez-Sanchez et al., 2012; Springman et al., 2014).

Three ST were identified, ST248, ST1, and ST22, found in 75, 15, and 10% of isolates, respectively, and in 81, 33, and 19% of herds; the 3 ST belong to distinct CC (i.e., ST248 to subCC103, ST1 to subCC1, and ST22 to subCC22). Figure 1 shows the population snapshot for the isolates obtained in this study and the existing *Strep. agalactiae* pubMLST database. Multiple clonally unrelated ST were identified in as many as 1 in 3 herds, and sometimes even within individual cows. Two cows were infected by 2 ST (i.e., ST248 in combination with ST1 or ST22), whereby the different ST were obtained on the same day but from different quarters. A total of 25, 5, and 3 cows were infected with a single ST (i.e., ST248, ST1, and ST22, respectively).

Presence of multiple ST of *Strep. agalactiae* within a dairy herd has also been reported in China, albeit at lower frequency (4 of 21 herds; Yang et al., 2013). By contrast, in northern Europe, only 1 ST was detected in most herds (Mahmmod et al., 2015; Jørgensen et al., 2016; Lyhs et al., 2016). If a second ST was detected in a herd in northern Europe, this would generally be a single locus variant of the dominant strain in that herd rather than a clonally unrelated ST (Mahmmod et al., 2015). Presence of multiple unrelated ST per herd, as observed in our study in Colombia, suggests introduction of *Strep. agalactiae* into the herd from multiple sources (e.g., via purchase of cattle). This phenomenon has previously been described for *Staphylococcus aureus*, where the number of strains per herd is associated with cattle importation practices (Middleton et al., 2002). In Denmark, introduction of *Strep. agalactiae* into herds was not linked to cattle movements, implying that a nonbovine source of the organism must exist (Mweu et al., 2014). In northern Europe, people were identified as a potential source of *Strep. agalactiae* for dairy cattle (Zadoks et al., 2011; Lyhs et al., 2016).

In addition to differences in within-herd heterogeneity, differences have been noted between countries in predominant ST. In a study from China, as in the current study from Colombia, subCC103 predominated (Yang et al., 2013). By contrast, subCC1, which was a minor CC in Colombia, dominated in Denmark, Finland, and Sweden (Zadoks et al., 2011; Lyhs et al.,

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