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Molecular characterization of non-*aureus Staphylococcus* spp. from heifer intramammary infections and body sites

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ABSTRACT

The purpose of this study was to investigate non-*aureus Staphylococcus* spp. intramammary infections (IMI) in periparturient heifers and determine the relationship of precalving body site isolation with precalving IMI and postcalving IMI using molecular speciation and strain-typing methods. Primiparous heifers were enrolled at approximately 14 d before expected calving date. Precalving mammary quarter secretions and body site swabbing samples (teat skin, inguinal skin, muzzle, and perineum) were collected. Postcalving, mammary quarter milk samples were collected for culture and somatic cell counting. Precalving body site samples were cultured, and up to 10 staphylococcal colonies were saved for characterization. Staphylococcal isolates were speciated using matrix-assisted laser/desorption ionization time-of-flight mass spectrometry or sequencing of *rpoB* or *tuf*. Pulsed-field gel electrophoresis was used to strain type a subset of isolates. Overall, *Staphylococcus chromogenes*, *Staphylococcus agnetis*, and *Staphylococcus simulans* were the most common species identified in precalving mammary secretions, whereas *S. chromogenes*, *Staphylococcus xylosus*, and *S. agnetis* were the most common species found in postcalving milk samples. The most common species identified from body site samples were *S. chromogenes*, *S. xylosus*, and *Staphylococcus haemolyticus*. Mammary quarters that had a precalving mammary secretion that was culture positive for *S. agnetis*, *S. chromogenes*, or *Staphylococcus devriesei* had increased odds of having an IMI with the same species postcalving. A *S. chromogenes* IMI postcalving was associated with higher somatic cell count when compared with postcalving culture-negative quarters. Among heifers identified with a non-*aureus Staphylococcus* spp. IMI either precalving or postcalving, heifers that had *S. agnetis* or *S. chromogenes* isolated from their teat skin had increased odds of having the same species found in their precalving mammary secretions, and heifers with *S. chromogenes*, *S. simulans*, and *S. xylosus* isolated from their teat skin precalving were at increased odds of having an IMI with the same species postcalving. Overall, 44% of all heifers with a *S. chromogenes* IMI around the time of parturition had the same strain isolated from a body site. Based on pulsed-field gel electrophoresis, a high level of strain diversity was found.
Key words: heifer, non-*aureus Staphylococcus* spp., matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, pulsed-field gel electrophoresis

INTRODUCTION

Non-*aureus Staphylococcus* spp. (**NAS**) are the most common cause of IMI in heifers both before and after calving (Fox et al., 1995; Fox, 2009; De Vliegher et al., 2012). The percentage of NAS-infected quarters in heifers postcalving is approximately 35 to 37% (Rajala-Schultz et al., 2009; Piepers et al., 2010; De Visscher et al., 2016a). When using molecular techniques to speciate NAS, the most common species identified in heifers include *Staphylococcus chromogenes*, *Staphylococcus simulans*, and *Staphylococcus xylosus* (De Visscher et al., 2016a), which are considered among the most important NAS species as they have a more substantial effect on udder health and milk quality than other NAS species (Supré et al., 2011; Fry et al., 2014). Mammary quarters infected at parturition with *S. chromogenes*, *S. simulans*, or *S. xylosus* can have a geometric mean SCC above 400,000 cells/mL (De Visscher et al., 2016a). Increased SCC early in the first lactation can have a lasting negative effect on milk quality and production, including elevations in first-lactation SCC (De Vliegher et al., 2004a) and decreased first-lactation milk produc-

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tion (Coffey et al., 1986; De Vliegher et al., 2005b). Furthermore, an increased SCC between 5 and 30 DIM of the first lactation can negatively affect lifetime milk yield (Archer et al., 2013). In general, mastitis in heifers has also been associated with increased culling, with each unit increase in the LnSCC in early lactation being associated with an 11% increase in culling hazard (De Vliegher et al., 2005a).

Staphylococci are part of the normal skin flora of animals (Devriese and De Keyser, 1980; Devriese et al., 1985) and have been isolated from different body sites of cows and heifers and from the dairy environment (White et al., 1989; Taponen et al., 2008; Piessens et al., 2011). With regard to isolation of NAS species from body sites, teat skin has been the primary focus of most studies (De Vliegher et al., 2003; Braem et al., 2013; De Visscher et al., 2016b). Studies to date report that *S. chromogenes*, followed by *Staphylococcus haemolyticus*, *Staphylococcus devriesei*, and *Staphylococcus equorum*, are the main NAS species isolated from teat ends of pregnant heifers (De Visscher et al., 2016b). Depending on the study, 20 to 62% of heifers harbor *S. chromogenes* on the teat skin (White et al., 1989; De Visscher et al., 2016b). Moreover, isolation of *S. chromogenes* from the teat end increases the likelihood of *S. chromogenes* IMI in the corresponding quarter at parturition (De Visscher et al., 2016a), which is similar to *Staphylococcus aureus* (Roberson et al., 1994). Although some research points to the negative effects of isolation of *S. chromogenes* from the teat end and its relationship to IMI, other studies have found a potential protective effect, as some strains display antibacterial activities in vitro (De Vliegher et al., 2004b; Braem et al., 2014), and heifer quarters with precalving isolation of *S. chromogenes* from the teat end had SCC <200,000 cells/mL in early lactation (De Vliegher et al., 2003).

Although ample evidence shows that some of the most important NAS species can be found on the teat end and in the mammary gland of early-lactation heifers, little work has been done to strain type NAS species identified on body sites of precalving heifers to determine whether the same strain is found in the mammary gland either before or after calving. Furthermore, little work has been done to characterize NAS species isolated from body sites other than the teat end and the relationship of such isolation to IMI. One study (White et al., 1989) described isolation of NAS species from body sites other than the teat end; however, that study did not explore the relationship between body site isolation and IMI and used phenotypic methods for speciation. Phenotypic speciation may lead to misclassification of some NAS species based on recent evidence on the poor typeability of phenotypic methods (Zadoks and Watts, 2009). Therefore, the purpose of this study was to investigate NAS IMI in periparturient heifers and determine the relationship of body site isolation, precalving NAS IMI, and postcalving NAS IMI using molecular speciation and pulsed-field gel electrophoresis (**PFGE**) strain typing.

MATERIALS AND METHODS

Herd and Heifer Selection

Late-gestation heifers (n = 100), including both Guernsey (n = 9) and Holstein (n = 91) breeds, at the University of Missouri Foremost Dairy Research Center (Columbia) were enrolled between April 2014 and April 2015. Heifers were enrolled in the study at approximately 14 d before expected calving date. During the study, the farm milked a median number of 179 (range: 171–193) Holsteins and 26 (range: 25–28) Guernsey cows, had a geometric mean bulk tank SCC of 206,000 cell/mL, and had an average 305-d milk production of 10,844 kg. All heifers included in the study were raised on the farm. Cows and heifers on the farm calved year-round with the exception of a planned reduction in calving from July to September to avoid calving during peak heat and humidity. The study was approved by the University of Missouri Animal Care and Use Committee (protocol no. 7815).

Sample Collection

Precalving mammary quarter secretions and postcalving mammary quarter milk samples were aseptically collected from the cohort of heifers. Prior to sample collection, teats were treated with a germicidal teat dip, hand stripped, dried with an individual towel, and then scrubbed with a cotton pad soaked in 70% isopropyl alcohol. Precalving secretion samples were collected approximately 14 d before expected calving date. All precalving mammary quarter secretion samples were taken after body site swabbing samples had been collected. In all cases, the person taking the samples wore disposal latex or nitrile gloves, and gloves were changed between animals. Postcalving mammary quarter foremilk samples were collected twice within the first 10 d of lactation for bacterial culture and somatic cell counting. Samples were collected twice weekly, with each sample collection being 3 to 4 d apart. Heifers were not sampled until they were at least 3 DIM. The median DIM for the first sample was 4 d (range: 3–7 d), and the median DIM for the second sample was 8 d (range: 6–10 d). All samples were chilled on ice for transport to the laboratory. Samples for bacterial culture were stored at −20°C until bacteriological examination was performed (median storage time: 32 d; range: 1–134