J. Dairy Sci. 101:1–13
https://doi.org/10.3168/jds.2017-13974

Cross-sectional study to identify staphylococcal species isolated from teat and inguinal skin of different-aged dairy heifers

P. R. F. Adkins,* S. Dufour,† J. N. Spain,‡ M. J. Calcutt,§ T. J. Reilly,§# G. C. Stewart,§‖ and J. R. Middleton*[1]
*Department of Veterinary Medicine and Surgery, University of Missouri, Columbia 65211
†Department of Pathology and Microbiology, University of Montreal, Quebec, Canada J2S 2M2
‡Department of Animal Science,
§Department of Veterinary Pathobiology,
#Veterinary Medical Diagnostic Laboratory, Department of Veterinary Pathobiology, and
‖Bond Life Sciences Center, University of Missouri, Columbia 65211

ABSTRACT

The purpose of this study was to describe the prevalence and distribution of staphylococcal species on the teat and inguinal skin of dairy heifers across the various stages of the heifer life cycle. The cross-sectional study included 106 Holstein heifers with an age range of 0 d to 27 mo that were selected from 11 different groups, based on housing type and age, on a single dairy operation. A composite swabbing sample including all 4 teats and a second composite sample including both inguinal regions of each heifer were collected using gas-sterilized electrostatic dusters (Swiffers; Procter and Gamble, Cincinnati, OH). Swabbing samples were mixed with 10 mL of sterile saline, agitated, and cultured on mannitol salt agar plates. At 24 h, plates were read and up to 10 staphylococcal colonies were saved for further analysis. Staphylococcal isolates were speciated using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or PCR amplification and partial sequencing of *rpoB* or *tuf*. The prevalence of staphylococci was compared between the inguinal and teat regions using the chi-squared or Fisher's exact test, as applicable. Logistic regression models were used to investigate the relationship between a heifer's age (treated as a quantitative continuous variable) and the probability of isolating a given staphylococcal species from a given body site (inguinal region or teats). Overall, the most common species identified were *Staphylococcus haemolyticus* followed by *Staphylococcus chromogenes*, *Staphylococcus xylosus*, *Staphylococcus devriesei*, and *Staphylococcus sciuri*. *Staphylococcus aureus* was more prevalent on the teat than in the inguinal region, whereas *Staphylococcus arlettae* was more prevalent in the inguinal region than on the teat. All other staphylococcal species were as likely to be found on the teat skin as the inguinal region skin. Isolation from the inguinal and teat skin was associated with age for *Staphylococcus agnetis*, *S. chromogenes*, *S. devriesei*, *Staphylococcus equorum*, *S. haemolyticus*, *Staphylococcus lentus*, *S. sciuri*, *Staphylococcus vitulinus*, *and S. xylosus*. The probability of finding *S. chromogenes* and *S. agnetis* on the teat and inguinal region increased with age, whereas the probability of *S. devriesei* and *S. haemolyticus* decreased with age. This study provides further insight into the ecology of staphylococcal species involved in heifer mastitis.

Key words: staphylococci, body site, heifer, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

INTRODUCTION

Non-*aureus Staphylococcus* (**NAS**) species are the most common cause of subclinical mastitis in dairy heifers around the time of parturition (Fox, 2009; De Vliegher et al., 2012). The true effect of NAS IMI is still under debate, with descriptions ranging from a protective effect against IMI with other mastitis pathogens to an association with mild clinical mastitis (Taponen et al., 2006; Piepers et al., 2013). Some of the variability in the effect of NAS on the mammary gland may be associated with species differences. Recent research using molecular identification methods has demonstrated diversity among NAS species that can be found on the dairy farm as well as differences between species with regard to IMI, associated mammary inflammation, persistence of IMI, and ecological niches on and off the cow (Piessens et al., 2011; Supré et al., 2011; De Visscher et al., 2014; Fry et al., 2014). Staphylococci have been found in abundance in various extramammary sites (defined as any site not inside the mammary gland) including environmental sites such as floor samples, air samples, and used bedding samples as well as bovine

Received October 10, 2017.
Accepted December 13, 2017.
[1]Corresponding author: middletonjr@missouri.edu

body sites including the perineum, udder skin, and teat skin (Taponen et al., 2008; Piessens et al., 2011; De Visscher et al., 2016b; P. R. F. Adkins, S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton, unpublished data).

With regard to the microbial population of body sites, the teat end has been the most thoroughly investigated (Braem et al., 2012, 2013; De Visscher et al., 2016b). The bacteria identified on the skin of the teat end include 4 bacterial phyla: *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* (Braem et al., 2012). The most frequently isolated genus of bacteria on the teat end is *Staphylococcus* (Braem et al., 2013). At the species level, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Staphylococcus devriesei*, and *Staphylococcus equorum* have been the predominant NAS species identified at the teat ends of pregnant heifers (De Visscher et al., 2016b). Identification of some staphylococcal species on the teat end has been found to be associated with IMI. Specifically, finding *S. chromogenes* on the teat skin of late-term heifers has been shown to significantly increase the odds of *S. chromogenes* IMI around the time of parturition (De Visscher et al., 2016a; P. R. F. Adkins, S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton, unpublished data). This finding has also been demonstrated for other host-adapted staphylococcal species, specifically *Staphylococcus aureus* (Roberson et al., 1994). Furthermore, in one study, 44% of all heifers with a *S. chromogenes* IMI around the time of parturition had the same strain of *S. chromogenes* isolated from a body site, with many originating from the teat skin (P. R. F. Adkins, S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton, unpublished data). Although *S. chromogenes* IMI has been linked to elevated milk SCC and persistent IMI (Supré et al., 2011; Fry et al., 2014), isolation of *S. chromogenes* from the prepartum teat end has been associated with an SCC of <200,000 cells/mL and may protect against IMI in early lactation (De Vliegher et al., 2003).

Few studies have looked at isolation of staphylococcal species from other body sites besides the teat end. One study, in which extramammary sites of lactating cows were evaluated, including the udder skin, teat ends, perineum, and streak canal, found *S. equorum*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, and *Staphylococcus xylosus* to be the predominant extramammary species (Taponen et al., 2008). In periparturient heifers, the teat skin, inguinal skin, muzzle, and perineum were evaluated and *S. chromogenes*, *S. xylosus*, *S. haemolyticus*, *S. sciuri*, and *S. devriesei* were found to be the predominant extramammary species (P. R. F. Adkins, S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton, unpublished data). Another study used phenotypic speciation methods to evaluate NAS species isolated from different-aged heifers' nares, hair coat, vagina, teat skin, and streak canal and found that *S. xylosus*, *S. chromogenes*, *Staphylococcus warneri*, and *S. sciuri* were the predominant species (White et al., 1989). Further, as heifers' age increased, the recovery of *S. xylosus* and *S. sciuri* decreased, whereas recovery of *S. chromogenes* and *S. warneri* increased (White et al., 1989). When specifically investigating *S. chromogenes* on heifers, De Vliegher et al. (2003) found that the chance of isolating the organism from at least 1 teat end increased significantly with age.

Changes in populations of staphylococcal species isolated from body sites as heifers age have not been examined using currently available molecular speciation methods. Previously used phenotypic identification methods were based on bacterial metabolic activity and morphologic features, which can be affected by culture conditions, subculturing, and storage (Sauer and Kliem, 2010). Also, interpretation of phenotypic tests can be subjective (Carretto et al., 2005). Therefore, it has been recommended that phenotypic speciation methods be replaced by molecular speciation methods when conducting studies on the epidemiology and ecology of NAS that cause bovine mastitis (Vanderhaeghen et al., 2015). Additionally, since the White et al. (1989) study, several new staphylococcal species have been named that have been isolated from dairy cattle or their environment, including *Staphylococcus nepalensis* (Spergser et al., 2003; Zadoks and Watts, 2009), *S. devriesei* (Supré et al., 2010), and *Staphylococcus agnetis* (Taponen et al., 2012). Furthermore, although the isolation of staphylococcal species from teat and inguinal skin in periparturient heifers has been recently described (P. R. F. Adkins, S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton, unpublished data), the relationship between the isolation of staphylococcal species on the teat skin and inguinal skin and heifer age has not been fully examined. Previous research investigating the isolation of *S. aureus* from body sites has specifically considered teat skin separately from the sides of the udder, and no *S. aureus* was found on udder sides (Matos et al., 1991). However, isolation of staphylococcal species from the inguinal region has not been evaluated among various heifer age groups. Therefore, the purpose of this study was to describe the prevalence and distribution of staphylococcal species on the teat and inguinal skin of dairy heifers by examining a cross-section of animals in various stages of the heifer life cycle on 1 farm.