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Teat apex colonization with coagulase-negative *Staphylococcus* species before parturition: Distribution and species-specific risk factors

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ABSTRACT

Coagulase-negative staphylococci (CNS) are the main cause of bovine intramammary infections and are also abundantly present in extramammary habitats such as teat apices. Teat apex colonization (TAC) with CNS has already been explored in lactating dairy cows at the species level, whereas this is not true for dry cows and end-term heifers. Therefore, the aim of this observational study was to describe CNS TAC in nonlactating dairy cows and end-term heifers in Flemish dairy herds and to identify associated risk factors at the herd, cow, and quarter level. All CNS were molecularly identified to the species level using transfer RNA intergenic spacer PCR (tDNA-PCR) and sequencing of the 16S rRNA gene, allowing for species-specific statistical analyses using multivariable, multilevel logistic regression. *Staphylococcus devriesei*, *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, and *Staphylococcus equorum* were the most frequently isolated species. *Staphylococcus chromogenes* was the sole species colonizing teat apices of cows and heifers in all herds, whereas large between-herd differences were observed for the other species. Teat apices of red and white Holstein Friesians, of quarters dried off without an internal teat sealer, and swabbed in months with lower precipitation and higher ambient temperature were significantly more likely to be colonized by *S. devriesei*. Slightly dirty teat apices and teat apices swabbed in months with lower precipitation had higher odds of being colonized by *S. chromogenes*, whereas teat apices sampled in months with lower precipitation and higher ambient temperature were more likely to be colonized by *S. haemolyticus*. Dirty teat apices and teat apices swabbed in months with lower ambient temperature in combination with low precipitation had higher odds of being colonized by *S. equorum*. Diverse factors explaining CNS TAC, yet mostly related to humidity, ambient temperature, and hygiene, substantiate differences in epidemiological behavior and ecology between species.
Key words: teat apex, coagulase-negative *Staphylococcus* species

INTRODUCTION

Besides being the most common cause of intramammary infections in dairy cows in many regions and countries (Vanderhaeghen et al., 2015), CNS are abundantly present in extramammary habitats such as sawdust and air (Piessens et al., 2011) and also colonize dairy cows' teat apices (Taponen et al., 2008; Braem et al., 2012; De Visscher et al., 2014). In fact, the majority of teat apices colonizing microbiota belong to the group of the CNS (Braem et al., 2013). Differences in ecological and epidemiological characteristics among bovine-associated CNS species have been revealed (Vanderhaeghen et al., 2015), yet many aspects remain undetermined. The presence of CNS on teat apices of lactating dairy cows and heifers has already been explored at the species level (Taponen et al., 2008; Braem et al., 2013; De Visscher et al., 2014; Vanderhaeghen et al., 2015) but little is known about CNS teat apex colonization (**TAC**) in dry cows and end-term heifers. A high prevalence of CNS colonized teat apices in pregnant dairy heifers before calving has been described (De Vliegher et al., 2003; Piepers et al., 2011), but those studies either solely concerned heifers, studied CNS as a group, or focused specifically on *Staphylococcus chromogenes* only using nonmolecular identification. Large observational studies describing the species distribution on teat apices in nonlactating dairy cows and end-term heifers and identifying factors associated with their presence are needed to add to our understanding of the role of CNS species in bovine udder health (Taponen et al., 2008; De Visscher et al., 2014; Vanderhaeghen et al., 2015). Although we have started to learn about the relation between CNS TAC and IMI and associations seem to be present (Leroy et al., 2015), potential protective aspects of TAC remain to be studied (Vanderhaeghen et al., 2014).

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Therefore, this study aimed to determine and to describe (1) the species-specific prevalence of CNS colonization of teat apices of dry cows and end-term heifers before calving; (2) the CNS distribution; (3) associated herd-, cow-, and quarter-level risk factors; and (4) the variance components of CNS TAC.

MATERIALS AND METHODS

Herds and Cows

Thirteen commercial Flemish dairy herds were included, all of them participating in the DHI program (CRV, Arnhem, the Netherlands), being a first selection criterion. Other inclusion criteria were no prepartum antibiotic treatment of heifers and the use of AI as accurate expected calving dates were demanded. The majority of farmers (n = 8) only housed dairy cattle, whereas the others also farmed pigs (n = 1) or beef cattle (n = 4). The DHI records and bulk milk quality data of the Milk Control Centre Flanders (Lier, Belgium) allowed for calculating the herd size and the bulk milk SCC, respectively, at the start of the sampling period in July 2012. The entire sampling period lasted until February 2013. Before the start of the study, an average of 57 cows and heifers were in lactation (range = 30–95) per herd (arithmetic mean of the 6 last test-day samples). The geometric mean bulk milk SCC was 201,000 cells/mL (range = 79,000–310,000 cells/mL). On 7 of the farms, dry cows were housed on concrete slatted floor with cubicles with mats (n = 4), mattresses (n = 2), or without bedding (n = 1). The other 6 farms kept the dry cows on straw, either in a deep litter barn (n = 5) or in deep litter boxes with a bottom layer of sand and a full concrete floor (n = 1). Pregnant heifers were typically housed on a concrete slatted floor in cubicles (n = 11) with mats (n = 5), with mattresses (n = 5), or without bedding (n = 1). The other 2 farms housed the pregnant heifers either in a deep litter barn with straw (n = 1) or in deep litter boxes with a bottom layer of sand and straw (n = 1). On the majority of farms, dry cows and pregnant heifers (n = 12 and n = 11, respectively) were kept on pasture between May and September. Seven of the herds housed the dry cows separated from the lactating cows before calving, whereas in the majority of herds (n = 10) the pregnant heifers were housed together with the lactating cows.

On each farm, 12 pregnant heifers and dry cows (total n = 156) were selected according to the proportion of lactating cows and heifers present in the herd at the start of the study, reflecting the parity distribution in the herd, resulting in a total of 53 pregnant heifers and 103 dry cows (range = start of second lactation to start of tenth lactation). The majority of the selected animals were black and white Holstein Friesian (**HF**; 85%, n = 132), and 15% were red and white HF (n = 24). Eighty-five percent (n = 132) of the animals were supplemented with minerals and vitamins before parturition. On all farms, blanket dry cow treatment was applied using either cloxacillin benzathine (47% of the 103 dry cows, n = 48 cows) or cephalosporins with a broad or gram-positive spectrum (40%, n = 41 cows and 10%, n = 10 cows, respectively). Dry cow treatment information was lacking for a few cows only (3%, n = 3) as these cows were purchased when dry. One cow did not receive antimicrobials at drying-off due to a sudden drop in milk production. Only a minority of the dry cows received an internal teat sealer (39% of the 103 dry cows, n = 40) at drying off. Iodine teat dip was applied to 38 dry cows and end-term heifers (24%) before parturition.

Samples and Data Collection

To determine TAC before parturition, swabs of all teat apices of the 153 cows and heifers (total n = 624) were collected 14 d before expected calving date. Visible soil and manure were first removed. Further, a dry cotton swab (Copan, Novolab, Belgium) was rotated gently on the teat apex as described by De Vliegher et al. (2003). Swabs were transported under cooled condition (4°C) to the Mastitis and Milk Quality Research Lab (Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium).

Several herd- and cow-level factors, potentially associated with CNS (species-specific) TAC, were either calculated based on DHI records or on data of the Milk Control Centre Flanders, or collected before the onset of the study via a questionnaire (Table 1). Other potential cow- and quarter-level factors were recorded at sampling (teat swabbing; Table 1).

Laboratory Analyses

All swabs were plated on mannitol salt agar (Oxoid, Erembodegem, Aalst, Belgium; one plate per swab; De Visscher et al., 2013). Plates were aerobically incubated at 37°C and examined after 24 and 48 h. One colony of all phenotypically different colony types was picked up and subcultured on esculin blood agar (Oxoïd; one quadrant per isolate) to obtain pure cultures for subsequent analysis. All potential CNS isolates were stored at −80°C or immediately identified to the species level using transfer RNA intergenic spacer PCR (**tDNA-PCR**). If no identification could be obtained, sequencing of the 16S rRNA gene was performed (Supré et al., 2009).