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Further evidence for the existence of environmental and host-associated species of coagulase-negative staphylococci in dairy cattle

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ABSTRACT

Coagulase-negative staphylococci (CNS) are abundantly present in the dairy farm environment and on bovine skin and mucosae. They are also the most prevalent bacteria causing bovine intramammary infections (IMI). Reservoirs and transmission routes of CNS are not yet fully unraveled. The objectives of this study were to explore the distribution of CNS in parlor-related extramammary niches and to compare it to the distributions of CNS causing IMI in those herds. Niches that were targeted in this study were cows' teat apices, milking machine unit liners, and milker's skin or gloves. Each of the three herds had its own CNS microbiota in those niches. The most prevalent species in the parlor-related extramammary niches were *Staphylococcus cohnii*, *S. fleurettii*, and *S. equorum* in the first, second, and third herd, respectively, whereas *S. haemolyticus* and *S. sciuri* were found in all herds. *S. cohnii* and *S. fleurettii*, as well as *S. haemolyticus*, which was present in each herd, were also frequently found in milk samples. By contrast, *S. chromogenes*, *S. simulans*, and *S. xylosus* favored the mammary gland, whereas *S. equorum* was more common in the parlor-associated niches. Within each herd, species distribution was similar between teat apices and milking machine unit liners. In conclusion, some of the extramammary niches related to the milking process might act as infection sources for IMI-causing CNS. This study provides further evidence that the group of CNS species is comprised of environmental, opportunistic and host-adapted species which differ in ecology.

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1. Introduction

Interest in coagulase-negative staphylococci (CNS) increased over the past years both in human (Rasmussen et al., 2000; Dekio et al., 2005) and in veterinary medicine (Taponen et al., 2007; Gillespie et al., 2009). Effort is ongoing to unravel the characteristics of CNS as a group (Sampimon et al., 2009; Piepers et al., 2010) and the specific characteristics of the different species (Simojoki et al., 2011; Supré et al., 2011). Recently, we reported that several CNS species were abundantly present in the cows' environment (air, slatted floor, and sawdust), but rarely associated with intramammary infections (IMI) (Piessens et al., 2011). These "environmental CNS" include *Staphylococcus equorum*, *S. fleurettii*, and *S. sciuri.* Other species, such as *S. haemolyticus* and *S. simulans*, were commonly found in the barn environment and were also isolated from quarters with IMI. These species are considered to be "opportunistic pathogens" (Piessens et al., 2011). *S. chromogenes* was commonly associated with IMI, but rarely found in the barn environment, implying that other reservoirs potentially play a role in its epidemiology (Piessens et al., 2011). *S. cohnii* and *S. xylosus* are also frequently isolated from infected quarters in some herds (Supré et al., 2011), but their sources are as yet unidentified.

The udder contains a natural barrier mechanism to separate and protect the mammary gland from the environment. Potential extramammary sources of infection that have not been examined in previous studies include the cows' teat apices (TA), the milking machine unit liners (MMUL) and milkers' skin. Previous work suggests that the presence of *S. aureus* on teat skin can be associated with the risk of infection in early lactation (Roberson et al., 1994), while milking machine unit liners also play a role in *S. aureus* transmission (Zadoks et al., 2002). Milkers' skin can carry *S. epidermidis*, which may cause IMI in cows (Thorberg et al., 2006). By contrast, presence of CNS (Piepers et al., 2011) and *S. chromogenes* (De Vliegher et al., 2003) on TA pre-partum was not associated with IMI (Piepers et al., 2011) and even associated with a decreased risk of *S. chromogenes* IMI in heifers in early lactation (De Vliegher et al., 2003). However, little is known about the distribution of CNS species on TA, MMUL, and milkers' skin or gloves (MSG) and their possible role as source of CNS IMI.

The aims of this study were (1) to map the distribution of the parlor-associated CNS species, based on a molecular identification technique, (2) to compare the distribution of parlor-associated species and CNS species causing IMI in these herds, and (3) to explore whether TA, MMUL, and the milker could play a role in CNS transmission in these herds.

2. Materials and methods

2.1. Herds, animals, and teat apices

A single cross-sectional sampling was performed in 3 commercial Flemish dairy herds in February 2008. The herds were part of a longitudinal study (starting in September 2007, ending in January 2009) to determine the impact of CNS species-specific IMI on quarter milk somatic cell count (Supré et al., 2011). Herd characteristics and udder health practices are described in Table 1.

Per herd, 25 animals were included in the longitudinal study on IMI and 6 of the 25 animals were randomly selected for the current study, with stratification by parity, i.e. 2 animals in their first, second, or higher lactation, respectively. Teat apices ($n = 72$) were sampled both pre- and post-milking, with the exception of one animal in herd 3 that was only sampled pre-milking ($n_{TA} = 140$). Sterile cotton swabs were used (Copan Diagnostics Inc., CA, USA). The sampling protocol was as follows: cleansing of the teat with an individual dry paper cloth, pre-milking swabbing of the teat apex, disinfection of the teats with alcohol, attachment of the milking cluster, milking, removal of the

Table 1
Overview of herd characteristics and udder health management practices of the three participating herds.

	Herd 1	Herd 2	Herd 3
Herd characteristics[a]			
Number of lactating cows	43.5 (39–48)[b]	48.9 (43–53)	65.7 (61–70)
Mean milk yield (kg/day)	30.9 (28.5–32.4)	32.3 (30.1–34.3)	31 (29.4–31.8)
Mean 305d-yield (in kg)	9921.6 (9551–10,189)	10,194.7 (9975–10,396)	10,180.2 (9978–10,367)
Herd SCC (×1000 cells/ml)[c]	152.9 (98–220)	195.9 (133–323)	186.7 (103–298)
Udder health management			
Gloves during milking	1 of 2 milkers	All milkers	1 of 2 milkers
Iodine post-milking teat disinfection	Dip	Dip	Spray
Dry-off management			
Long-acting antimicrobial	Cloxacillin	Cefquinome	Nafcillin/penicillin/streptomycin
Internal teat sealer	Yes	Yes	Yes
Routine culling of chronically infected cows	No	Yes	No
Calving pen			
Separate straw box	Yes	Yes	Yes
Used for sick cows	Yes	No	Yes
Bedding material	Sawdust	Sawdust	Sawdust
Cleaning of slatted floor	2× per day	Not regularly	2× per day

[a] Measured through the Dairy Herd Improvement (DHI) program.
[b] (Minimum – maximum).
[c] Average of the monthly herd somatic cell count (SCC), measured through the DHI program.