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Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health

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ABSTRACT

Coagulase-negative staphylococci (CNS) are the main cause of bovine intramammary infections (IMI) in many countries. Despite a high prevalence of CNS IMI at parturition, species-specific risk factor studies, relying on accurate identification methods, are lacking. Therefore, this observational study aimed at determining the prevalence and distribution of different CNS species causing IMI in fresh heifers and dairy cows in Flemish dairy herds and identifying associated species- and subgroup-specific risk factors at the herd, cow, and quarter level. The effect on udder health was investigated as well. *Staphylococcus chromogenes*, *S. sciuri*, and *S. cohnii* were the most frequently isolated species. The only CNS species causing IMI in fresh heifers and dairy cows in all herds was *Staphylococcus chromogenes*, whereas large between-herd differences in distribution were observed for the other species. Quarters from heifers and quarters with an inverted teat end had higher odds of being infected with *S. chromogenes*, *S. simulans*, or *S. xylosus* as well as with *S. chromogenes* solely. Prepartum teat apex colonization with *S. chromogenes* increased the likelihood of *S. chromogenes* IMI in the corresponding quarters at parturition. Quarters with dirty teat apices before calving were more likely to be infected with *S. cohnii*, *S. equorum*, *S. saprophyticus*, or *S. sciuri*, supporting the environmental nature of these CNS species. Three species (*S. chromogenes*, *S. simulans*, and *S. xylosus*) were associated with a higher quarter somatic cell count at parturition as compared with uninfected quarters.
Key words: dairy cattle, mastitis, coagulase-negative staphylococci, risk factor

INTRODUCTION

Coagulase-negative staphylococci are the most prevalent cause of bovine IMI in many countries and are predominantly found in milk samples of fresh heifers (De Vliegher et al., 2012). Recent work has shown that CNS are, in many aspects, not a homogeneous group (Vanderhaeghen et al., 2014, 2015). Because *Staphylococcus chromogenes* is the most prevalent species causing bovine IMI and is rarely isolated from the bovine environment (Piessens et al., 2011, 2012), a host-adapted nature is assumed. In turn, *Staphylococcus cohnii*, *Staphylococcus equorum*, *Staphylococcus saprophyticus*, and *Staphylococcus sciuri* are more commonly present in environmental habitats (Piessens et al., 2011) than in milk (Piessens et al., 2011; Supré et al., 2011; Fry et al., 2014), indicating an environmental ecology. Furthermore, *S. chromogenes*, *Staphylococcus simulans*, and *Staphylococcus xylosus* have a more substantial effect on udder health than other species. They can cause a considerable increase in quarter SCC (Supré et al., 2011; Fry et al., 2014). Species-specific distribution of CNS in fresh cows and heifers from different Flemish dairy herds has not yet been described.

Despite the high prevalence of CNS IMI, only a few studies have focused on the identification of associated risk factors. One recent study identified CNS group-specific predictors using molecular identification, yet solely concerned CNS IMI throughout lactation (De Visscher et al., 2015). Other studies only included fresh heifers (Piepers et al., 2011; Verbeke et al., 2012; Passchyn et al., 2014) or were conducted at the CNS group level (Sampimon et al., 2009; Piepers et al., 2011; Verbeke et al., 2012; Passchyn et al., 2014). The need exists to identify species-specific risk factors for CNS IMI. However, large studies are required to reach a sufficient number of isolates per species for this purpose. In the absence of adequate numbers, it is defensible to create subgroups of species; for example, based on a common effect on udder health, a common ecological nature, or a common epidemiological behavior. This approach at least circumvents studying CNS as a group, as was commonly done in earlier studies, and respects recent findings indicating that CNS are not a homogeneous group.

This observational study aimed at (1) determining the species-specific prevalence and distribution of CNS

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IMI in fresh heifers and cows in Flemish dairy herds, (2) assessing the variance components of subgroup- and species-specific IMI, and (3) identifying associated subgroup- and species-specific herd-, cow-, and quarter-level risk factors. In addition, (4) the effect on the quarter milk SCC in early lactation was studied for several species.

MATERIALS AND METHODS

Herd and Cows

Thirteen commercial Flemish dairy herds were selected by convenience and included in this observational study. Herd inclusion criteria were (1) participation in the DHI program in Flanders on an annual basis with an interval of 4 to 6 wk between 2 test-days (CRV, Arnhem, the Netherlands), (2) no prepartum antibiotic treatment of heifers, and (3) the use of AI to predict the expected calving date as accurately as possible. On each farm, 12 end-term heifers and dry cows per herd (total n = 156) were randomly selected in accordance with the proportion of lactating heifers and cows at the start of the study period (July 2012), resulting in a total of 53 end-term heifers and 103 dry cows. The total study period lasted until February 2013. Detailed herd and cow information can be found elsewhere (De Visscher et al., 2016).

Samples and Data Collection

Within 4 d after parturition, quarter milk samples (total n = 624) were aseptically collected following the guidelines of the National Mastitis Council (Hogan et al., 1999) for bacteriological culturing, and quarter milk SCC (**qSCC**) was determined. Milk samples were transported under cooled conditions (4°C) to the Mastitis and Milk Quality Research Laboratory (Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium). Quarter milk SCC were immediately determined using a Direct Cell Counter (DCC, DeLaval, Gent, Belgium).

Various herd- and cow-level risk factors potentially associated with CNS subgroup- or species-specific IMI at parturition were recorded or collected via a questionnaire at the start of the study period (July 2012) (Table 1). The DHI records allowed us to calculate the herd size. On average, there were 57 (range = 30–95) milking cows and heifers per herd (arithmetic mean of the 6 last test-day samples) at the start of the sampling period. Categorization of the herd size was based on the median value of all calculated aforementioned arithmetic means: smaller herds (i.e., <60 lactating animals) and larger herds (i.e., ≥60 lactating animals). Bulk milk SCC were available through the bulk milk quality data of the Milk Control Centre Flanders (MCC Flanders, Lier, Belgium) and recoded into lower bulk milk SCC (i.e., <200,000 cells/mL) and higher bulk milk SCC (i.e., ≥200,000 cells/mL) according to Schukken et al. (2009).

At each sampling of fresh cows and heifers (i.e., within 4 d after calving), other potential cow- and quarter-level variables were recorded (Table 1). The Royal Meteorological Institute of Belgium records monthly ambient temperature (°C) and precipitation (L/m^2). Classification of temperature and precipitation was based on the median of all monthly values during the study period (from July 2012 to February 2013); that is, 10°C and 59.35 L/m^2, respectively. Scoring of teat apex condition was performed based on a visual scoring system (Neijenhuis et al., 2000) and recoded afterward into a good condition, a protuberant teat end, or an inverted teat end. Teat skin condition was scored visually into either "little grooves" (i.e., normal, smooth, soft, healthy, shallow grooves) or "many grooves" (i.e., more dry, rough and with deeper grooves). Fourteen days before expected calving date, swabs of teat apices (n = 624) were collected.

Laboratory Analyses

All quarter milk samples (n = 624) were plated on mannitol salt agar (**MSA**; Oxoid, Aalst, Belgium; 1 quadrant per milk sample) and aerobically incubated at 37°C (De Visscher et al., 2013) to recover CNS. Plates were examined after 24 and 48 h. All phenotypically different colony types were counted, and 1 colony per colony type was picked up and subcultured on esculin blood agar (1 quadrant per colony; Oxoid) to obtain pure cultures. All potential CNS isolates were stored at −80°C for subsequent analysis or immediately identified to the species level using transfer RNA intergenic spacer PCR (**tDNA-PCR**) or sequencing of the 16S rRNA gene if no identification was obtained (Supré et al., 2009). All quarter milk samples were additionally plated on esculin blood agar (Oxoid) and MacConkey agar (Oxoid) and examined according to the guidelines of the National Mastitis Council (Hogan et al., 1999). This information was used only to exclude quarters also infected with any major pathogen (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, other esculin-positive streptococci, *Escherichia coli*, *Klebsiella*, *Pseudomonas*, yeast and molds) from the subsequent statistical analyses.

All teat swabs were plated on MSA as described by De Visscher et al. (2016) and examined as described above to identify the CNS species colonizing teat apices before parturition.

Table 1. Overview of herd-, cow-, and quarter-level variables investigated as risk factors for IMI with (subgroups of) CNS at parturition in a cohort of 53 heifers and 103 dairy cows in 13 Flemish dairy herds

Independent variable	Recording method	Description	Breakdown categories in final model
Herd level			
Herd size	DHI records	Mean number of cows in lactation at start of sampling period[1]	Smaller (<60 lactating cows) vs. larger (≥60 lactating cows) herd[2]
Bulk milk SCC	MCC records[3]	Mean bulk milk SCC at start of sampling period[4]	Lower bulk milk SCC (<200 × 10^3 cells/mL) vs. higher bulk milk SCC (≥200 × 10^3 cells/mL)[5]
Cow level			
Housing	Questionnaire	Housing of a specific dry cow or pregnant heifer	Cubicles vs. deep litter
Pasture access	Questionnaire	Pasture access during outdoor season	No pasture vs. pasture
Contact	Questionnaire	Contact with lactation cows before parturition	No contact vs. contact
Breed	DHI records	Breed	Black and white HF[6] vs. red and white HF
Parity	DHI records	Parity starting at parturition	Second lactation or older vs. first lactation
Vitamins	Questionnaire	Supplementation with minerals and vitamins before parturition	No supplementation vs. supplementation
Antimicrobials	Questionnaire	Antimicrobials used at drying-off	No vs. narrow-spectrum vs. broad-spectrum[7]
Teat sealer	Questionnaire	Application of an internal teat sealer at drying-off	No teat sealer vs. teat sealer
Teat disinfection	Questionnaire	Teat dipping or spraying before parturition	No dipping/spraying vs. dipping/spraying
Calving pen	Questionnaire	On straw or on pasture	Straw vs. pasture
Ease of calving	Questionnaire	Progress of calving	Unassisted vs. easy pull vs. hard pull
BCS	Visual	Five-point scale[8] at sampling	<2.5 vs. 2.5–3 vs. >3
Hygiene	Visual	Hygiene of mammary gland and teats at sampling[9]	Very clean vs. slightly dirty vs. dirty
Temperature	RMI[10]	Monthly ambient temperature (°C) at sampling of a specific dry cow or pregnant heifer	Low vs. high[11]
Precipitation	RMI	Monthly precipitation (L/m^2) at sampling of a specific dry cow or pregnant heifer	Low vs. high[11]
Quarter level			
Quarter position	Visual	Position of the quarter	Front vs. hind quarter
Teat apex condition	Visual	Teat apex condition at sampling	Good condition vs. protuberant teat end vs. inverted teat end[12]
Teat skin condition	Visual	Teat skin condition at sampling	Little grooves vs. many grooves[13]
Teat apex colonization	Swabbing and genotypic analysis[14]	Species-specific teat apex colonization 14 d before expected calving date	Colonized with a certain CNS species vs. colonized with another CNS species vs. not colonized

[1]Arithmetic mean based on 6 last DHI records before the start of the study (July 2012).

[2]Categorization based on median value of all mean values of all herds.

[3]Milk Control Centre Flanders (Lier, Belgium).

[4]Geometric mean based on 6 last records of the Milk Control Centre Flanders before the start of the study.

[5]Categorization based on Schukken et al. (2009).

[6]Holstein Friesian.

[7]Antimicrobials with a narrow spectrum include cloxacillin, benzathine, cefalexine, and cefalonium, whereas antibiotics with a broad spectrum only include cefquinome.

[8]Edmonson et al. (1989).

[9]Categorization based on a 5-point scale (Reneau et al., 2005).

[10]Royal Meteorological Institute of Belgium (Brussels).

[11]Categorization based on median value of all monthly records from 1 yr (March 2012 to February 2013); that is, 10°C and 59.35 L/m^2.

[12]Categorization based on a visually scoring system of Neijenhuis et al. (2000) and recoded afterward.

[13]Teat skin condition was scored visually into "little grooves" (i.e., normal, smooth, soft, healthy, shallow grooves) and "many grooves" (i.e., more dry, rough and with deeper grooves).

[14]De Visscher et al. (2016).

Defining Quarter IMI Status

Quarters were considered infected with a specific CNS species when ≥1 cfu/0.01 mL of milk of this species was observed on MSA, according to the definition of Dohoo et al. (2011), to maximize the sensitivity. Quarters infected with ≥3 genotypically different CNS species or with (a) major pathogen(s), were excluded from the subsequent statistical analyses.

Descriptive and Risk Factor Analysis

The prevalence and herd-specific distribution of all CNS species at parturition was first computed. Before analyses were performed, observations were checked for unlikely values. Complete data were available from 608 quarters of 99 fresh cows and 53 fresh heifers. Dry-cow treatment information of 3 cows of the same herd was missing. The cows were purchased by the current owner during their dry period. Also, some data were missing due to loss of one questionnaire. However, the cow belonged to a different herd than the 3 other cows of which the dry cow treatment data were lacking.

Logistic multilevel regression models were fit (MLwiN 2.16, Centre for Multilevel Modeling, University of Bristol, Bristol, UK) as follows:

$$Y_{ijk} \sim \text{Binomial } (\pi_{ijk}),$$

$$\text{Logit } (\pi_{ijk}) = \beta_{0ijk} + \beta_1 X_{ijk} + v_{0k} + u_{ojk},$$

$$\text{Var } (Y_{ijk} \mid \pi_{ijk}) = \pi_{ijk}(1 - \pi_{ijk}) \text{ with } v_{0k} \sim \text{Normal } (\mu_k, \sigma_k) \text{ and } u_{ojk} \sim \text{Normal } (\mu_{jk}, \sigma_{jk}),$$

where Y_{ijk} is the infectious status of quarter i from cow j and herd k, and π_{ijk} the probability of this quarter (1) being infected at parturition with *S. chromogenes*, *S. simulans*, or *S. xylosus*, the so-called more relevant CNS species, or uninfected, or infected with another CNS species; (2) being infected or not at parturition with the so-called host-adapted species *S. chromogenes*; and (3) being infected or not at parturition with a so-called environmental species; that is, *S. cohnii*, *S. equorum*, *S. saprophyticus*, or *S. sciuri.* Y_{ijk} is a function of the explanatory variable X through the logit function, and approximately follows a binomial distribution; β_{0ijk} is the intercept; that is, the baseline probability of infection when all predictors are equal to zero; β_1 is the regression coefficient for explanatory variable X; v_{0k} and u_{ojk} are the herd and cow random effects, respectively, and approximately follow normal distributions with respective variances σ_k and σ_{jk}. Reweighted iterative generalized least squares and first-order penalized quasi-likelihood estimation methods were used to estimate these models.

The proportion of variation for the outcome variables at the herd, cow, and quarter levels was first estimated using null hierarchical models (i.e., models with only random effects and no fixed predictors). The variance at the quarter level was assumed $\pi^2/3$, as described by Goldstein et al. (2002).

Then, univariable models examining associations between the outcome variables and the independent variables (potential risk factors, see Table 1) were fit to select variables associated with each outcome. Statistical significance was assessed at $P < 0.15$. Afterward, Spearman correlation coefficients among the statistically significant variables were calculated to identify multi-collinearity in the multivariable models. One out of 2 variables was selected, according to biological relevance, for further analysis if a correlation coefficient $\geq|0.6|$ was calculated. Next, multivariable models were fit for the significant variables from the univariable analysis using backward stepwise elimination with statistical significance assessed at $P < 0.05$. Biologically relevant interaction terms were tested between all remaining statistically significant risk factors and kept in the final multivariable model when significant ($P < 0.05$). Also, confounding was investigated using classic epidemiological criteria. A factor was considered a confounder if its removal caused a relative change >25% in the regression coefficients of the remaining variables or with a regression coefficient between −0.4 and 0.4 if and absolute change >0.1 was observed (Noordhuizen et al., 2001).

To test the fitness of all final models, the observational-level standardized residuals were plotted against the observational-level predicted values. Additionally, Hosmer-Lemeshow goodness-of-fit tests were assessed on the fixed effect models only (version 9.3, SAS Institute Inc., Cary, NC; Dohoo et al., 2009). The test was never statistically significant, indicating good fit of our models.

Odds ratios (**OR**) and the 95% CI were calculated to present the magnitude of the associations.

Effect on Quarter Milk SCC

A linear mixed regression model was fit (MLwiN 2.16, Centre for Multilevel Modeling) to determine the association between subgroups of CNS species and the natural log-transformed qSCC, as follows:

$$Y_{ijk} = \beta_{0ijk} + \beta_1 X_{1ijk} + \beta_2 X_{2ijk} + v_{0k} + u_{ojk} + \varepsilon_{ijk},$$

where Y_{ijk} is the natural log-transformed qSCC of quarter i from cow j and herd k; Y_{ijk} is a function of the explanatory variables X_1 and X_2 and approximately follows a normal distribution; X_1 is the fixed effect of the infectious status of the quarter (3 levels: uninfected, infected with the less-relevant CNS, infected with the more-relevant CNS species; i.e., *S. chromogenes*, *S. simulans*, or *S. xylosus*); X_2 is the fixed effect to adjust for the day of sampling (3 levels: first day, second day, third day or later); β_{0ijk} is the intercept (overall mean); β_1 and β_2 are the regression coefficients for explanatory variables X_1 and X_2, respectively; v_{0k} and u_{ojk} are the herd and cow random effects, respectively, and approximately follow normal distributions with respective variances σ_k and σ_{jk}; and ε_{ijk} is the random error term, assumed to be normally distributed with mean 0 and variance σ^2. Reweighted iterative generalized least squares were used to estimate the model. Quarters infected with a major pathogen were excluded from this data set.

RESULTS

Distribution

Thirty-four percent (n = 211 out of 624 quarters) of all quarter milk samples collected at parturition yielded growth on MSA. Per plate, 0 to 5 phenotypically different colony types were present. After tDNA-PCR or sequencing of the 16S rRNA gene, several different colony types represented the same CNS species, resulting in 19 different species and 191 CNS isolates available for further analysis.

Twenty-six percent of all quarters (n = 163 of 624 quarters) were infected at parturition with 1 or 2 different CNS species. *Staphylococcus chromogenes* (13% of all quarters and 41% of all isolates; n = 79) was the predominant species, followed by *S. sciuri* (4 and 13%, respectively; n = 25), *S. cohnii* (3 and 11%, respectively; n = 20), *S. equorum* (2 and 7%, respectively; n = 14), *S. xylosus* (2 and 7%, respectively; n = 13), and *S. haemolyticus* (1 and 5%, respectively; n = 9). Phenotypic or genotypic identification revealed 84 non-CNS-isolates on MSA belonging to the phyla *Firmicutes* (*Aerococcus* spp., n = 3; *Bacillus* spp., n = 43; *Jeotgallicoccus* spp., n = 1; *Staphylococcus aureus*, n = 5; *Streptococcus* spp., n = 22) and *Proteobacteria* (*Pseudomonas*, n = 8; *Psychrobacter*, n = 2).

In each herd, between 3 (herd 9) and 10 (herd 6) different CNS species were isolated. The only CNS species causing IMI at parturition in all herds was *S. chromogenes*. *Staphylococcus sciuri* and *S. xylosus* were infecting quarters in 10 herds, whereas *S. cohnii* and *S. equorum* both caused IMI in 7 herds. *Staphylococcus haemolyticus* could only be isolated from IMI in 6 herds. Other species were only causing IMI on a minority of farms (Table 2 and Figure 1).

The majority of fresh heifers (74%, n = 39 of 53 heifers) were diagnosed with a CNS IMI at parturition in at least one quarter, whereas only half of the multiparous cows were infected with CNS (46%, n = 47 out of 103 multiparous cows). Thirty-seven percent (n = 79 of 212 quarters) and 20% (n = 84 of 412 quarters) of all quarters of heifers and older cows, respectively, had a CNS IMI. *Staphylococcus hyicus* only caused IMI in quarters from heifers whereas *S. auricularis*, *S. capitis*, *S. devriesei*, *S. hominis*, *S. lentus*, *S. pasteuri*, *S. vitulinus*, and *S. warneri* were only isolated from IMI from quarters from multiparous cows (Table 2). All aforementioned species were, however, only rarely present in quarter milk at parturition. A considerably higher percentage of heifers had quarters infected with the more-relevant CNS (*S. chromogenes*, *S. simulans*, and *S. xylosus*; 60% heifers, n = 32 out of 53 heifers and 28% quarters, n = 60 out of 212 quarters) as opposed to multiparous cows (27% cows, n = 28 out of 103 older cows and 8% quarters, n = 35 out of 412 quarters). An almost equal number of heifers and multiparous cows harbored an environmental species (30% heifers, n = 16 out of 53 heifers and 11% quarters, n = 23 out of 212 quarters vs. 28% cows, n = 29 out of 103 older cows and 9% quarters, n = 38 out of 212 quarters). In contrast, *S. chromogenes* was predominantly isolated from quarters from heifers compared with multiparous cows (51% heifers, n = 27 of 53 heifers and 25% quarters, n = 53 of 212 quarters versus 19% cows, n = 20 of 103 older cows and 6% quarters, n = 26 of 412 quarters; Table 2).

Risk Factors

For "IMI with relevant species" and "IMI caused by *S. chromogenes* only," no variation occurred at the herd level, whereas for "IMI with the environmental species," variation was observed at all 3 levels in the null model (Table 3).

Fitting the univariable models revealed 7 cow-level and 2 quarter-level risk factors to be associated with IMI at parturition both with the more relevant species as well as with *S. chromogenes* only. Only 3 cow-level factors were unconditionally associated with IMI with the so-called environmental species (Table 4). The risk factors "antimicrobials" (reflecting whether narrow- or broad-spectrum antibiotics were used at drying off) and "parity" were correlated (Spearman rho = 0.85). The latter was expected as no antibiotics were administered to end-term heifers, which is in contrast with the multiparous cows, where all except one cow received

Table 2. Species distribution (number and %) of CNS causing IMI at parturition isolated from 624 quarters from 53 fresh heifers (H) and 103 fresh multiparous cows (C) in 13 Flemish dairy herds

	Herd 1		Herd 2		Herd 3		Herd 4		Herd 5		Herd 6		Herd 7		Herd 8		Herd 9		Herd 10		Herd 11		Herd 12		Herd 13		Total	
Species	C[1]	H[2]	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	N	%
S. chromogenes	6.3	18.8	3.6	20.0	8.3	25.0		31.3	3.6	30.0	11.1	25.0	3.1	25.0	6.3	18.8	5.6	16.7	10.7	20.0	6.3	56.3	12.5	12.5	5.0	25.0	79	41.4
S. sciuri	3.1		3.6	25.0	4.2				7.1	10.0	5.6	16.7	6.3	6.3		12.5	2.8				3.1				2.5	12.5	25	13.1
S. cohnii	12.5		7.1	5.0	4.2	4.2	15.6			5.0	8.3								3.6	5.0							20	10.5
S. equorum	3.1	12.5							7.1		5.6		6.3		3.1				3.6	10.0	6.3						14	7.3
S. xylosus				5.0		4.2	3.1	6.3	7.1				3.1	6.3		6.3	2.8			5.0	6.3				2.5		13	6.8
S. haemolyticus		6.3	3.6	5.0			3.1				2.8		3.1										6.3	6.3			9	4.7
S. arlettae				5.0									9.4	12.5													6	3.1
S. simulans	3.1						3.1								3.1					5.0					12.5		5	2.6
S.	3.1											8.3	6.3														4	2.1
saprophyticus																												
S. devriesei											2.8		3.1						3.6								3	1.6
S. vitulinus							3.1				2.8																2	1.0
S. epidermidis															3.1								6.3				2	1.0
S. hominis							6.3																				2	1.0
S. lentus	6.3																										2	1.0
S. auricularis													3.1														1	0.5
S. capitis																					3.1						1	0.5
S. hyicus					4.2																						1	0.5
S. warneri											2.8																1	0.5
S. pasteuri											2.8																1	0.5

[1]Percentage of quarters of fresh cows infected with a specific species within each herd.
[2]Percentage of quarters of fresh heifers infected with a specific species within each herd.

antibiotics at drying off. Only "parity" was used in the subsequent models.

Table 5 presents results from the final multilevel, multivariable logistic regression models. Quarters from heifers were more likely to be infected with the more-relevant species and with *S. chromogenes*, representing the host-adapted species, as opposed to multiparous cows (OR = 3.9; 95% CI: 2.2–7.0 and OR = 4.2; 95% CI: 2.1–8.3, respectively). Quarters with an inverted teat end had increased odds of being infected with the more relevant species and solely *S. chromogenes* compared with quarters with a good teat end condition (OR

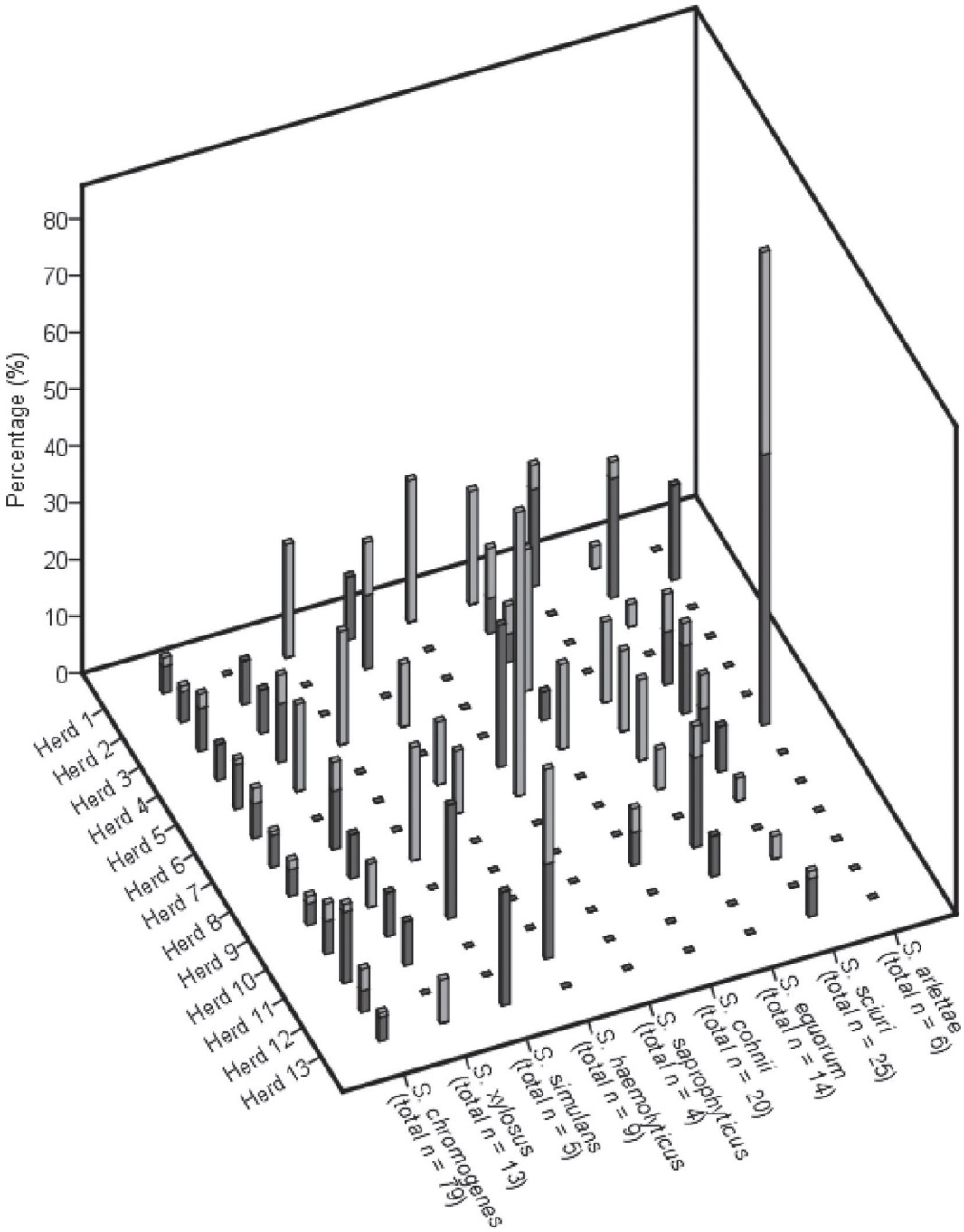

Figure 1. Species distribution of the most frequently isolated CNS causing IMI at parturition in 13 Flemish dairy herds: percentages of quarters positive for a certain CNS species per herd among the total number of quarters positive for a certain CNS species are shown. Percentages are divided according to the proportion of IMI from fresh heifers (dark gray) and fresh dairy cows (light gray), respectively, caused by a certain species per herd.

Table 3. Variance components at the herd, cow, and quarter levels of the null models for IMI with (subgroups of) CNS species[1]

	S. chromogenes, *S. simulans*, *S. xylosus* (n = 95)			*S. chromogenes* (n = 79)[1]			*S. cohnii*, *S. equorum*, *S. saprophyticus*, *S. sciuri* (n = 61)		
Level	Var. est.[2]	SE	%	Var. est.	SE	%	Var. est.	SE	%
Herd	0.00	0.00	0.0	0.00	0.00	0.0	0.30	0.25	6.9
Cow	1.23	0.38	27.2	1.73	0.51	34.5	0.78	0.44	17.8
Quarter	3.29	—	72.8	3.29	—	65.5	3.29	—	75.3
Total variance	4.52		100	5.02		100	4.37		100

[1]*S. chromogenes*, *S. simulans*, and *S. xylosus* represent the CNS species more relevant for udder health; *S. chromogenes* is representative for the host-adapted species, and *S. cohnii*, *S. equorum*, *S. saprophyticus*, and *S. sciuri* represent environmental species.
[2]Variance components.

Table 4. Univariable, multilevel logistic regression models[1] for IMI at parturition with (subgroups of) CNS species[2]

	S. chromogenes, *S. simulans*, *S. xylosus* (n = 95)					*S. chromogenes* (n = 79)					*S. cohnii, S. equorum, S. saprophyticus, S. sciuri* (n = 61)				
Independent variable	N_{nCNS}[3]	N_{CNS}[4]	OR[5]	95% CI		N_n[6]	N_{CNS}	OR	95% CI		N_n	N_{CNS}	OR	95% CI	
Herd level															
Herd size															
Smaller	244	44	Ref.			214	40	Ref.			214	27	Ref.		
Larger	285	51	0.99	0.55	1.8	247	39	0.83	0.42	1.7	247	34	1.2	0.47	3.0
Bulk milk SCC															
Lower	280	56	Ref.			241	47	Ref.			241	34	Ref.		
Higher	249	39	0.82	0.45	1.5	220	32	0.77	0.39	1.5	220	27	0.80	0.32	2.0
Cow level															
Housing															
Cubicles	328	72	Ref.*			280	60	Ref.*			280	46	Ref.*		
Deep litter	201	23	0.53	0.28	1.0	181	19	0.52	0.25	1.1	181	15	0.50	0.22	1.1
Pasture access															
No	44	16	Ref.*			32	15	Ref.*			32	9	Ref.		
Yes	485	79	0.44	0.18	1.1	429	64	0.31	0.11	0.86	429	52	0.46	0.14	1.5
Contact															
No	246	30	Ref.*			215	25	Ref.*			215	26	Ref.		
Yes	283	65	1.9	1.8	2.0	246	54	1.8	0.91	3.7	246	35	1.2	0.58	2.5
Breed															
Black and white HF[7]	447	81	Ref.			384	66	Ref.			384	58	Ref.*		
Red and white HF	82	14	0.91	0.40	2.1	77	13	0.90	0.35	2.3	77	3	0.27	0.07	1.1
Parity															
≥Second lactation	377	35	Ref.*			328	26	Ref.*			328	38	Ref.		
First lactation	152	60	4.3	2.4	7.6	133	53	5.0	2.6	10.0	133	23	1.5	0.75	2.8
Vitamins															
No	85	11	Ref.			77	9	Ref.			77	8	Ref.		
Yes	444	84	1.4	0.58	3.2	384	70	1.4	0.54	3.8	384	53	1.4	0.38	5.0
Antimicrobials[8]															
No	156	60	Ref.*			137	53	Ref.*			137	23	Ref.		
Narrow spectrum	215	17	0.20	0.10	0.41	187	13	0.17	0.08	0.40	187	24	0.69	0.33	1.5
Broad spectrum	148	16	0.28	0.13	0.59	128	11	0.23	0.09	0.56	128	13	0.73	0.29	1.8
Teat sealer[8]															
No	373	79	Ref.*			330	65	Ref.*			330	42	Ref.		
Yes	146	14	0.44	0.21	0.95	122	12	0.52	0.21	1.2	122	18	1.1	0.46	2.4
Teat disinfection															
No	404	68	Ref.			351	56	Ref.			351	49	Ref.		
Yes	125	27	1.3	0.68	2.57	110	23	1.4	0.65	3.0	110	12	0.91	0.37	2.3
Calving pen															
Straw	512	92	Ref.			448	76	Ref.			448	61	Ref.		
Pasture	17	3	1.0	0.19	5.29	13	3	1.6	0.26	10.0	13		—[9]	—	—
Ease of calving															
Unassisted	260	40	Ref.*			231	36	Ref.			231	25	Ref.		
Easy pull	204	32	1.1	0.56	2.07	175	25	0.96	0.45	2.1	175	27	1.3	0.65	2.6
Hard pull	65	23	2.4	1.0	5.3	55	18	2.1	0.81	5.6	55	9	1.4	0.50	3.8

Continued

Table 4 (Continued). Univariable, multilevel logistic regression models[1] for IMI at parturition with (subgroups of) CNS species[2]

	S. chromogenes, *S. simulans*, *S. xylosus* (n = 95)					*S. chromogenes* (n = 79)					*S. cohnii*, *S. equorum*, *S. saprophyticus*, *S. sciuri* (n = 61)				
Independent variable	N_{nCNS}[3]	N_{CNS}[4]	OR[5]	95% CI		N_n[6]	N_{CNS}	OR	95% CI		N_n	N_{CNS}	OR	95% CI	
BCS[10]															
<2.5	45	7	Ref.			35	7	Ref.			35	9	Ref.		
2.5–3	453	83	1.1	0.38	3.4	397	68	0.80	0.24	2.7	397	51	—	—	—
>3	28	4	0.84	0.15	4.8	27	4	0.63	0.09	4.3	27	0	—	—	—
Hygiene[10]															
Very clean	70	6	Ref.			68	4	Ref.*			68	2	Ref.*		
Slightly dirty	222	34	1.7	0.57	5.2	197	29	2.3	0.61	8.4	197	21	1.2	0.47	3.0
Dirty	234	54	2.6	0.88	7.6	194	46	0.90	0.45	1.8	194	37	6.4	1.3	30.9
Temperature															
Low	84	20	Ref.			72	17	Ref.			72	13	Ref.		
High	445	75	0.74	0.35	1.6	389	62	0.72	0.30	1.8	389	48	0.71	0.30	1.7
Precipitation															
Low	385	71	Ref.			338	59	Ref.			338	42	Ref.		
High	144	24	0.87	0.44	1.7	123	20	0.92	0.42	2.0	123	19	1.4	0.66	2.8
Quarter level															
Quarter position															
Front	261	51	Ref.			230	41	Ref.			230	28	Ref.		
Hind	268	44	0.83	0.52	1.3	231	38	0.92	0.55	1.5	231	33	1.2	0.66	2.0
Teat end condition[10]															
Good	461	64	Ref.*			403	51	Ref.*			403	47	Ref.		
Protuberant	10	3	1.8	0.37	9.0	9	3	1.8	0.31	10.5	9	0	—	—	—
Inverted	55	27	3.6	0.45	1.5	47	25	4.2	1.8	9.8	47	13	—	—	—
Teat skin condition[10]															
Little grooves	300	51	Ref.			266	43	Ref.			266	28	Ref.		
Many grooves	226	43	1.1	0.63	2.1	193	36	1.2	0.60	2.3	193	32	1.4	0.70	2.7
Teat apex colonization															
Not colonized	152	24	Ref.*			133	20	Ref.*			133	22	Ref.		
Colonized with another species[11]	274	40	0.96	0.52	1.8	261	32	0.89	0.44	1.8	218	25	0.56	0.28	1.1
Colonized with the same species[12]	103	31	1.9	0.98	3.8	67	27	3.0	1.3	6.6	110	14	0.66	0.29	1.5

[1]Cow and herd included in all models as random effects to correct for potential clustering of quarters within cows and cows within herds.

[2]*S. chromogenes*, *S. simulans,* and *S. xylosus* represent the CNS species more relevant for udder health; *S. chromogenes* is representative of host-adapted species, and *S. cohnii*, *S. equorum*, *S. saprophyticus*, and *S. sciuri* represent environmental species.

[3]Number of quarters uninfected with CNS or infected with species other than the one of the subgroup at parturition.

[4]Number of IMI at parturition caused by different subgroups of CNS, respectively.

[5]Odds ratios are presented; Ref. = reference category per risk factor.

[6]Number of quarters uninfected with CNS at parturition.

[7]Holstein Friesian.

[8]Missing data from 3 dry cows.

[9]Model did not converge due to too low numbers.

[10]Missing data from 1 dry cow.

[11]Colonized with species other than the ones of the subgroups, respectively.

[12]Colonized with species of the different subgroups, respectively.

*Overall *P*-value of the risk factor (all categories compared with the Ref. category) is <0.15.

= 2.8; 95% CI: 1.4–5.9 and OR = 3.7; 95% CI: 1.5–8.8, respectively). Quarters with teat apices colonized with *S. chromogenes* before calving had higher odds of being infected at parturition with this same species (OR = 3.3; 95% CI: 1.4–7.5). Quarters with dirty teat apices were more likely to be infected with an environmental CNS species (OR = 6.4; 95% CI: 1.3–30.9) compared with quarters with clean teats. In contrast, IMI with *S. chromogenes*, representing the host-adapted species, were not significantly associated with the hygiene of the teats.

Post Hoc Power Calculation

A post hoc power calculation was conducted at the quarter level first. The more-relevant species, the host-adapted species, and the so-called environmental species were frequently isolated subgroups of CNS species: approximately 61 to 95 quarters were infected with those subgroups. With 61 positive quarters and 461 control quarters (the model concerning the environmental species), an association corresponding to an OR of 2.0 ($\alpha = 0.05$), assuming 20 and 50% exposure among

Table 5. Final multivariable, multilevel logistic regression models[1] describing herd-, cow- and quarter-level risk factors associated with IMI at parturition with (subgroups of) CNS species[2]

	Staphylococcus chromogenes, *S. simulans*, *S. xylosus* (n = 95)					*Staphylococcus chromogenes* (n = 79)					*Staphylococcus cohnii*, *S. equorum*, *S. saprophyticus*, *S. sciuri* (n = 61)				
Independent variable	β[3]	SE	OR[4]	95% CI	*P*-value[5]	β	SE	OR	95% CI	*P*-value	β	SE	OR	95% CI	*P*-value
Intercept	−2.62	0.22			<0.001	−3.08	0.38			<0.001	−3.64	0.78			<0.001
Herd level[6]															
Cow level															
Parity					<0.001					<0.001					
≥ Second lactation	Ref.[7]	—	—	—	—	Ref.	—	—	—	—					
First lactation	1.37	0.30	3.9	2.2	7.0	1.43	0.35	4.2	2.1	8.3					
Hygiene															0.05
Very clean											Ref.	—	—	—	—
Slightly dirty											1.37	0.81	3.9	0.80	19.4
Dirty											1.85	0.81	6.4	1.3	30.9
Quarter level															
Teat end condition					0.02					0.01					
Good	Ref.	—	—	—	—	Ref.	—	—	—	—					
Protuberant	0.44	0.82	1.6	0.31	7.7	0.66	0.92	1.9	0.32	11.8					
Inverted	1.04	0.38	2.8	1.4	5.9	1.30	0.45	3.7	1.5	8.8					
Teat apex colonization										<0.01					
Not colonized						Ref.	—	—	—	—					
Colonized with another species[8]						−0.02	0.38	0.99	0.47	2.1					
Colonized with the same species[9]						1.18	0.43	3.3	1.4	7.5					

[1]Cow and herd included in all models as random effects to correct for potential clustering of quarters within cows and cows within herds.

[2]*Staphylococcus chromogenes*, *S. simulans*, and *S. xylosus* represent CNS species more relevant for udder health; *S. chromogenes* is representative for the host-adapted species, and *S. cohnii*, *S. equorum*, *S. saprophyticus,* and *S. sciuri* represent the environmental species.

[3]Regression coefficient.

[4]OR = odds ratio.

[5]*P*-value for the overall effect.

[6]No herd-level variables were identified.

[7]Ref. = referent category per risk factor.

[8]Colonized with species other than the ones of the subgroups, respectively.

[9]Colonized with species of the different subgroups, respectively.

controls, was detected with 64.6 and 69.7% power, respectively. With 95 positive quarters and 529 control quarters (the model concerning the more-relevant species), an association corresponding to an OR of 2.0 ($\alpha = 0.05$), assuming 20 and 50% exposure among controls, was detected with 79.8 and 86.2% power, respectively (Sampsize software, http://sampsize.sourceforge.net/iface/s3.html).

Second, a post hoc power calculation was performed at the cow level. Approximately 43 to 57 cows and heifers were infected with the subgroups of the environmental and more-relevant CNS species, respectively. With 43 animals being infected and 109 control animals (the model concerning the environmental species), an association corresponding to an OR of 2.0 ($\alpha = 0.05$), assuming 20 and 50% exposure among controls, was detected with 41.6 and 45.7% power, respectively, whereas with 57 animals being infected and 95 control animals (the model concerning the more-relevant species), the power was 45.6 and 52.1%, respectively (Sampsize software, http://sampsize.sourceforge.net/iface/s3.html).

Clustering of observations was not taken into account for these calculations, potentially overestimating the power.

Quarter Milk SCC

The geometric mean qSCC at parturition were 218 $\times 10^3$ cells/mL (range = 8 $\times 10^3$ to 5,596 $\times 10^3$ cells/mL), 173 $\times 10^3$ cells/mL (range = 20 $\times 10^3$ to 5,887 $\times 10^3$ cells/mL) and 442 $\times 10^3$ cells/mL (range = 37 $\times 10^3$ to 5,093 $\times 10^3$ cells/mL) for the uninfected quarters (n = 409), the quarters infected with the less-relevant CNS (n = 58), and the quarters infected with the more-relevant CNS species (*S. chromogenes*, *S. simulans*, and *S. xylosus*; n = 86), respectively. Quarters infected at parturition with the more relevant CNS species had a significantly higher qSCC compared with uninfected quarters (i.e., 528 $\times 10^3$ cells/mL vs. 240 $\times 10^3$ cells/mL (LSM = 6.3 vs. LSM = 5.5). The qSCC at parturition of quarters infected with a less-relevant CNS species was not different from the qSCC of uninfected quarters (i.e., 235 $\times 10^3$ cells/mL vs. 240 $\times 10^3$ cells/mL (LSM = 5.5 vs. LSM = 5.5; Table 6).

Table 6. Final multilevel linear regression model[1] describing subgroups of CNS species at parturition associated with the natural log-transformed quarter SCC

Independent variable	β[2]	SE	95% CI		*P*-value[3]
Intercept	5.77	0.16			<0.001
Infectious status[4]					<0.001
Noninfected	Ref.				
Less relevant CNS	−0.03	0.15	0.72	1.3	
More relevant CNS	0.79	0.14	1.7	2.9	
Days in milk					<0.001
1st day	Ref.				
2nd day	−0.15	0.23	0.55	1.4	
3rd day or later	−0.72	0.18	0.34	0.69	

[1]Cow and herd included in the model as random effects to correct for potential clustering of quarters within cows and cows within herds.

[2]Regression coefficient; Ref. = reference category.

[3]*P*-value for the overall effect.

[4]*S. chromogenes*, *S. simulans*, and *S. xylosus* represent the CNS species more relevant for udder health; CNS species other than those 3 are considered less relevant for udder health.

DISCUSSION

This large observational study describes the species-specific prevalence and distribution of CNS IMI immediately after parturition in both heifers and multiparous cows in several Flemish dairy herds and substantiates the relevance for udder health of some of the CNS species. For the first time, potentially associated subgroup- and species-specific risk factors for IMI at calving were investigated. Including a large number of quarters and the use of molecular speciation provide valuable and precise information on CNS IMI in fresh cows and heifers and adds to the existing knowledge on the ecology and epidemiology of bovine-associated CNS.

The percentage of CNS-infected quarters of fresh heifers (37%) approached the 36% (Rajala-Schultz et al., 2004) and 35% (Piepers et al., 2010) of previous studies, exceeded the 10% of Compton et al. (2007), but was lower than the high prevalence of 74% reported by Taponen et al. (2007). In the latter study, however, samples were collected on the day of calving. The prevalence of CNS-infected quarters of multiparous cows (20%) was almost identical to the 16% (Rajala-Schultz et al., 2004) and 20% (Taponen et al., 2007) reported by other research groups.

The most prevalent species at parturition was *S. chromogenes*, confirming earlier reports (Taponen et al., 2007; Rajala-Schultz et al., 2009). *Staphylococcus sciuri* was the second most frequently isolated species, but was rarely isolated in previous genotypic work, collecting milk samples within 2 wk after calving (Fry et al., 2014). The same was true for *S. equorum*. *Staphylococcus cohnii*, *S. xylosus*, and *S. haemolyticus* were commonly identified by the current study and by Fry et al. (2014). We rarely observed *S. simulans* and *S. epidermidis*, which might be related to the limited number of herds in our study (n = 13) compared with the Fry et al. (2014) study (n = 89) or to a country- or region-dependent CNS microbiota.

We observed no between-herd variation in the null hierarchical models when investigating IMI with the relevant species (mainly *S. chromogenes*) and with *S.*

chromogenes only, which can be explained by the fact that *S. chromogenes* caused IMI in all herds. In contrast, variation existed both between cows and between herds in the null model with IMI caused by environmental species as outcome variable, suggesting at least some more variation in management practices between the herds. A greater number of herds and a random, instead of convenient, selection procedure could have resulted in more diverse management styles and could have increased the variation residing at the herd level.

Our post hoc power calculation indicated that at the cow level, only a strong association would be significantly different from 1. A higher number of animals included in the study could have resulted in a larger number of significant cow-level risk factors.

Supplementation with vitamins, ease of calving (Passchyn et al., 2014), and teat dipping before parturition (Piepers et al., 2011) have been associated with the likelihood of CNS IMI in heifers, but were not important in the current study. The same was true for pasturing during the outdoor season (Sampimon et al., 2009). On the other hand, housing can influence hygiene, which is associated with increasing odds of CNS IMI in heifers at parturition (Piepers et al., 2011). The latter observation again reinforces the environmental ecology of *S. cohnii*, *S. equorum*, *S. saprophyticus*, and *S. sciuri*, and emphasizes the value of studying CNS at the subgroup or species level. The more-relevant species were significantly more frequently observed in milk from quarters with an inverted teat end. Teat end shape has previously been linked to a higher prevalence of IMI (Seykora and McDaniel, 1985). Milk deposits on the teat end most likely provide a good growth substrate for bacteria, and a larger diameter of the streak canal, associated with inverted teats, allows easier access for bacteria.

Teat apex colonization with *S. chromogenes* significantly increased the odds of *S. chromogenes* IMI at parturition, a phenomenon that was not observed for the other species (data not shown). The latter illustrates the host-adapted nature of *S. chromogenes*—teat apices might act as a habitat for host-adapted species. This has been suggested before for *S. aureus* IMI at parturition (Roberson et al., 1994) and for *S. chromogenes* IMI throughout lactation (Taponen et al., 2008). An earlier study from our group focusing on *S. chromogenes* but not using molecular speciation did not demonstrate this link (De Vliegher et al., 2003). To reach better conclusions, strain typing of all isolates should be performed and is currently ongoing.

It has previously been shown that CNS-infected quarters have a higher SCC compared with negative control quarters (Taponen et al., 2007; Gillespie et al., 2009; Schukken et al., 2009). However, recent research reported an effect on the qSCC depending on the CNS species involved (Sampimon et al., 2009; Thorberg et al., 2009; Simojoki et al., 2011). Our findings confirm this variation between species. In fact, the more-relevant species (*S. chromogenes*, *S. simulans*, and *S. xylosus*) induced a higher qSCC at parturition, as has been reported previously (Supré et al., 2011; Fry et al., 2014; De Visscher et al., 2015). We also confirmed that quarters infected with species other than the so-called relevant ones have a qSCC that is not different from that of uninfected quarters (Supré et al., 2011; Fry et al., 2014). A limitation of CNS research is the fact that sensitivity can reach a maximum of 86.7% (Dohoo et al., 2011), indicating that some quarters classified as negative can be CNS infected. The latter might explain the high qSCC of the negative quarters observed in this study. However, to avoid an effect of a major pathogen on the qSCC, all quarters infected with a major pathogen were removed from the data set.

CONCLUSIONS

Staphylococcus chromogenes, *S. sciuri*, and *S. cohnii* were the predominant species causing IMI in fresh heifers and dairy cows. The only CNS species isolated from milk in all herds was *S. chromogenes*; the presence of other species differed by herd. The environmental nature was supported for *S. cohnii*, *S. equorum*, *S. saprophyticus,* and *S. sciuri*, whereas the host-adapted nature of *S. chromogenes* was substantiated. Prepartum teat apex colonization with *S. chromogenes* increased the likelihood of *S. chromogenes* IMI in the corresponding quarters at parturition. The more relevant species increased quarter SCC at parturition compared with uninfected quarters.

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REFERENCES

Compton, C. W. R., C. Heuer, K. Parker, and S. McDougall. 2007. Epidemiology of mastitis in pasture-grazed peripartum dairy heifers and its effects on productivity. J. Dairy Sci. 90:4157–4170.

De Visscher, A., F. Haesebrouck, S. Piepers, W. Vanderhaeghen, K. Supré, F. Leroy, E. Van Coillie, and S. De Vliegher. 2013. Assessment of the suitability of mannitol salt agar for growing bovine-associated coagulase-negative staphylococci and its use under field conditions. Res. Vet. Sci. 95:347–351.

De Visscher, A., S. Piepers, F. Haesebrouck, and S. De Vliegher. 2016. Teat apex colonization with coagulase-negative *Staphylococcus* species before parturition: distribution and species-specific risk factors. J. Dairy Sci. 99:1427–1439.

De Visscher, A., S. Piepers, K. Supré, F. Haesebrouck, and S. De Vliegher. 2015. Short communication. Species group-specific predictors at the cow and quarter level for intramammary infection with coagulase-negative staphylococci in dairy cattle throughout lactation. J. Dairy Sci. 98:5448–5453.

De Vliegher, S., L. K. Fox, S. Piepers, S. McDougall, and H. W. Barkema. 2012. Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. J. Dairy Sci. 95:1025–1040.

De Vliegher, S., H. Laevens, L. A. Devriese, G. Opsomer, J. L. M. Leroy, H. W. Barkema, and A. de Kruif. 2003. Prepartum teat apex colonization with *Staphylococcus chromogenes* in dairy heifers is associated with low somatic cell count in early lactation. Vet. Microbiol. 92:245–252.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Mixed models for discrete data. Pages 579–606 in Veterinary Epidemiologic Research. I. Dohoo, W. Martin, and H. Stryhn, ed. 2nd ed. AVC Inc., Charlottetown, Canada.

Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. and Mastitis Research Workers' Conference. 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. J. Dairy Sci. 94:250–261.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72:68–78.

Fry, P. R., J. R. Middleton, S. Dufour, J. Perry, D. Scholl, and I. Dohoo. 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. J. Dairy Sci. 97:4876–4885.

Gillespie, B. E., S. I. Headrick, S. Boonyayatra, and S. P. Oliver. 2009. Prevalence and persistence of coagulase-negative *Staphylococcus* species in three dairy research herds. Vet. Microbiol. 134:65–72.

Goldstein, H., R. W. Browne, and J. Rasbash. 2002. Partitioning variation in multilevel models. Underst. Stat. 4:223–231.

Hogan, J. S., R. N. Gonzáles, R. J. Harmon, S. C. Nickerson, S. P. Oliver, J. W. Pankey, and K. L. Smith. 1999. Laboratory Handbook on Bovine Mastitis. Rev. ed. National Mastitis Council, Madison, WI.

Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. T. M. Noordhuizen. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. J. Dairy Sci. 83:2795–2804.

Noordhuizen, J. P. T. M., K. Frankena, M. V. Thursfield, and E. A. M. Graat. 2001. Application of Quantitative Methods in Veterinary Epidemiology. Wageningen Pers, Wageningen, the Netherlands.

Passchyn, P., S. Piepers, and S. De Vliegher. 2014. Pathogen group-specific risk factors for intramammary infection in treated and untreated dairy heifers participating in a prepartum antimicrobial treatment trial. J. Dairy Sci. 97:6260–6270.

Piepers, S., G. Opsomer, H. W. Barkema, A. de Kruif, and S. De Vliegher. 2010. Heifers infected with coagulase-negative staphylococci in early lactation have fewer cases of clinical mastitis and a higher milk production in their first lactation than non-infected heifers. J. Dairy Sci. 93:2014–2024.

Piepers, S., K. Peeters, G. Opsomer, H. W. Barkema, K. Frankena, and S. De Vliegher. 2011. Pathogen group specific risk factors at herd, heifer and quarter levels for intramammary infections in early lactating dairy heifers. Prev. Vet. Med. 99:91–101.

Piessens, V., S. De Vliegher, B. Verbist, G. Braem, A. Van Nuffel, L. De Vuyst, M. Heyndrickx, and E. Van Coillie. 2012. Characterization of coagulase-negative *Staphylococcus* species from cows' milk and environment based on *bap, icaA*, and *mecA* genes and phenotypic susceptibility to antimicrobials and teat dips. J. Dairy Sci. 95:7027–7038.

Piessens, V., E. Van Coillie, B. Verbist, K. Supré, G. Braem, A. Van Nuffel, L. De Vuyst, M. Heyndrickx, and S. De Vliegher. 2011. Distribution of coagulase-negative *Staphylococcus* species from dairy cows' milk and environment differs between herds. J. Dairy Sci. 94:2933–2944.

Rajala-Schultz, P. J., K. L. Smith, J. S. Hogan, and B. C. Love. 2004. Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. Vet. Microbiol. 102:33–42.

Rajala-Schultz, P. J., A. H. Torres, F. J. DeGraves, W. A. Gebreyes, and P. Patchanee. 2009. Antimicrobial resistance and genotypic characterization of coagulase-negative staphylococci over the dry period. Vet. Microbiol. 134:55–64.

Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Associations between hygiene scores and somatic cell scores in dairy cattle. J. Am. Vet. Med. Assoc. 227:1297–1301.

Roberson, J. R., L. K. Fox, D. D. Hancock, and J. M. Gay. 1994. Ecology of *Staphylococcus aureus* isolated from various sites on dairy farms. J. Dairy Sci. 77:3354–3364.

Sampimon, O. C., H. W. Barkema, I. M. G. A. Berends, J. Sol, and T. J. G. M. Lam. 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134:37–44.

Schukken, Y. H., R. N. González, L. L. Tikofsky, H. F. Schulte, C. G. Santisteban, F. L. Welcome, G. J. Bennett, M. J. Zurakowski, and R. N. Zadoks. 2009. CNS mastitis: Nothing to worry about? Vet. Microbiol. 134:9–14.

Seykora, A. J., and B. T. McDaniel. 1985. Udder and teat morphology related to mastitis resistance. A review. J. Dairy Sci. 68:2087–2093.

Simojoki, H., T. Salomäki, S. Taponen, A. Iivanainen, and S. Pyörälä. 2011. Innate immune response in experimentally induced bovine intramammary infection with *Staphylococcus simulans* and *S. epidermidis*. Vet. Res. 42:49.

Supré, K., S. De Vliegher, O. C. Sampimon, R. N. Zadoks, M. Vaneechoutte, M. Baele, E. De Graef, S. Piepers, and F. Haesebrouck. 2009. Technical note: Use of transfer RNA-intergenic spacer PCR combined with capillary electrophoresis to identify coagulase-negative *Staphylococcus* species originating from bovine milk and teat apices. J. Dairy Sci. 92:3204–3210.

Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De Vliegher. 2011. Some coagulase-negative *Staphylococcus* species affect udder health more than others. J. Dairy Sci. 94:2329–2340.

Taponen, S., J. Björkroth, and S. Pyörälä. 2008. Coagulase-negative staphylococci isolated from bovine extramammary sites and intramammary infections in a single dairy herd. J. Dairy Res. 75:422–429.

Taponen, S., J. Koort, J. Björkroth, H. Saloniemi, and S. Pyörälä. 2007. Bovine intramammary infections caused by coagulase-negative staphylococci may persist throughout lactation according to amplified fragment length polymorphism-based analysis. J. Dairy Sci. 90:3301–3307.

Thorberg, B.-M., M.-L. Danielsson-Tham, U. Emanuelson, and K. Persson Waller. 2009. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J. Dairy Sci. 92:4962–4970.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, and S. De Vliegher. 2014. Invited review. Effect, persistence, and virulence of coagulase-negative *Staphylococcus* species associated with ruminant udder health. J. Dairy Sci. 97:5275–5293.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, and S. De Vliegher. 2015. Identification, typing, ecology and epidemiology of coagulase-negative staphylococci associated with ruminants. Vet. J. 203:44–51.

Verbeke, J., S. Piepers, L. Peelman, M. Van Poucke, and S. De Vliegher. 2012. Pathogen-group specific association between CXCR1 polymorphisms and subclinical mastitis in dairy heifers. J. Dairy Res. 79:341–351.