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Quarter- and cow-level risk factors for intramammary infection with coagulase-negative staphylococci species in Swiss dairy cows

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ABSTRACT

Bacteriological status, evaluation of udder symmetry, udder hygiene, and teat end scores of 92 dairy cows were assessed on 3 Swiss dairy farms in a longitudinal 1-yr study to determine risk factors for intramammary infection (IMI) with coagulase-negative staphylococci (CNS) species. Farm visits were performed monthly including sterile quarter milk sampling and udder evaluation of all lactating cows. Milk samples were evaluated for the presence of staphylococci using selective agar plates. Species identification was performed using MALDI-TOF mass spectrometry. Intramammary infection was defined as milk samples having ≥100 cfu per mL of milk according to culture results. Overall, 3,151 quarter samples were included in the statistical analysis. *Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Staphylococcus xylosus*, and a *Staphylococcus warneri*-like species were the 4 most prevalent CNS species found. Hierarchical multivariable logistic regression models were built to evaluate risk factors for species-specific CNS IMI. Risk factors for *Staph. chromogenes* IMI were presence in herd B, the period from June 2014 to August 2014 and December 2014 to February 2015, and presence of udder edema. For *Staph. haemolyticus*, the relevant risk factor included coinfection with *Staph. xylosus* coinfection with other than the above-mentioned CNS species ("others") and the period from June 2014 to November 2014. Coinfection with *Staph. haemolyticus* and "others," the periods from June 2014 to August 2014 and December 2014 to February 2015, early phase of lactation (1–60 d in milk), and belonging to herd B were significantly associated with *Staph. xylosus* IMI. Mid and late lactation, coinfection with *Staph. xylosus*, and the period September 2014 to May 2015 were identified as significant risk factors for *Staph. warneri*-like IMI. For *Staph. chromogenes*, 60.6 and 26% of the variance was observed at the quarter and cow level, respectively, whereas for the other investigated species the highest variance was observed at the sample level. The predominant species within herds differed and was most pronounced for the *Staph. warneri*-like species.
Key words: udder health, species level, subclinical mastitis, minor pathogen

INTRODUCTION

Species of the CNS group are commonly found in bovine milk samples (Tenhagen et al., 2006; Schukken et al., 2009; Thorberg et al., 2009). Subclinical IMI caused by CNS are highly prevalent in European dairy herds (Roesch et al., 2007; Sampimon et al., 2009; Rüegsegger et al., 2014), including herds with a high bulk milk SCC (Piepers et al., 2007). With improved control of major mastitis pathogens, minor pathogens, such as CNS, are coming more into focus as causative agents of increased SCC (Supré et al., 2011; Tomazi et al., 2015).

Various studies investigated risk factors for infection with CNS as a group at the herd level. A lower CNS prevalence was observed in Canadian herds with sand- or wood-based bedding products and in herds where cows had access to pasture (Dufour et al., 2012). In contrast, Sampimon et al. (2009) found increased odds for CNS IMI when cows had access to pasture in Dutch dairy herds. Other identified manageable herd-level risk factors included dry cows housed in one group instead of multiple groups, the use of non-tap water as a drinking source, and a high percentage of stalls contaminated with milk after milk leakage (Sampimon et al., 2009). In heifers, increased odds for CNS IMI were found in animals with a poor hygiene score, lack of teat dipping, and nonclipped udders before calving (Piepers et al., 2011). Prevalence of CNS IMI was shown to be higher in milk samples from primiparous than multiparous cows (Matthews et al., 1992; Tenhagen et al., 2006; Sampimon et al., 2009). An increased risk to be infected with the relevant species *Staphylococcus chromogenes*,

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Staphylococcus simulans, and *Staphylococcus xylosus* was found for heifers compared with multiparous cows (De Visscher et al., 2015).

Differences between causative species exist (Thorberg et al., 2009) and different CNS species may have different pathogenicity and persistence abilities within the udder (Supré et al., 2011). Grouping of CNS species was suggested for less prevalent species and performed based on other authors' observations on the effect of these species on udder health (Supré et al., 2011; Fry et al., 2014). A very recent study investigated risk factors for IMI in fresh cows at the species level, grouping species according to their relevance and ecology (De Visscher et al., 2016). The results showed that relevant risk factors were mainly found on the cow and quarter level for *Staph. chromogenes* but remain unclear for other species because those were grouped.

The objective of this study was to determine quarter and cow level risk factors for the presence of IMI caused by selected, prevalent CNS species during a period of 1 yr in 3 closed commercial dairy herds.

MATERIALS AND METHODS

The study was approved by the committee for animal experimentation (KTV) of the Canton of Bern, Switzerland (BE58/14).

Herd Characteristics

Three commercial Swiss dairy herds (A, B, and C) with known presence of CNS were selected from herds participating in the herd health service of the Clinic for Ruminants, University of Bern. The selection criterium "presence of CNS" was determined with data from regular herd health visits as follows: within the framework of this service all cows with an individual composite SCC >150,000 cells/mL (regular monthly milk recording) were examined by CMT and quarters scoring >1 were sampled for bacteriological analysis. Based on the criteria above, all herds had a known presence of CNS as a group (A = 35.7%, B = 42.9%, C = 33.3%) in the year 2013 and no history of a herd problem with major mastitis pathogens. Additional selection criteria included the willingness of the farmers to participate and the availability of digital DHI records. On farm A and B, cows were housed in freestall barns with straw-bedded cubicles, whereas cows on farm C were housed in a tiestall bedded on rubber mats covered with sawdust or straw. Cows in all 3 herds grazed on pasture during the vegetation period (April to October) and calved all year round. On all farms, milking was performed twice a day by one milker; on farm A in a 1 × 4 parallel parlor milking system, on farm B in a 1 × 3 herringbone milking parlor, and on farm C with a pipeline milking system. For udder preparation (i.e., teat cleaning), all farmers used at least 1 disposable disinfection towel (i.e., paper towel drenched in chloramine solution) per cow. Postmilking teat disinfection with iodine-based products was performed routinely on farm A and sporadically on farm B. On Farm C no postmilking teat disinfection was performed. During the study period, no significant changes in milking routine and the milking systems were introduced, except for the milker on farm C, who started to wear disposable gloves during the winter months. Selective dry cow therapy was carried out by all 3 farmers using different antimicrobial preparations including penicillin, amoxicillin, cloxacillin, and neomycin based on the cows' history, SCC, and bacteriological culture results. Low SCC cows without a history of mastitis were dried off without applying an antimicrobial treatment or by applying an internal teat sealant (Orbeseal, Zoetis Schweiz GmbH, Zürich, Switzerland).

Data Collection

This longitudinal study was carried out from June 2014 to May 2015. Herds were visited every 4 weeks during milking time to collect cow level information and aseptic single quarter milk samples from all lactating cows. Herd visits were conducted 13 times in each herd by 4 trained persons. Antibiotic treatment in case of clinical mastitis or at drying off was reported by the farmers. Cow level information such as breed, parity, calving date, milk yield, and milk solids (fat, protein, and urea) were gathered from the regular (monthly) milk recordings performed by the breeding association (Swiss Herdbook, Zollikofen, Switzerland). The time of the year the sampling was performed was categorized according to the chronology of sampling as June to August 2014, September to November 2014, December 2014 to February 2015, and March to May 2015.

Farmers were allowed to change herd management practices during the study. Bacteriological results were communicated to the farmers when available. A description of the collected data is summarized in Table 1.

Sampling Procedure

After teat cleaning and premilking of at least 3 streams of milk by the farmers, teats were thoroughly disinfected with cotton swabs drenched with ethanol (70%). Individual quarter foremilk samples were then aseptically collected, and latex gloves were worn. Gloves

Table 1. Description of covariates and their corresponding categories evaluated in statistical analyses of data on IMI with CNS on the species level in 3 commercial Swiss dairy herds

Item	Categorization
Cow level	
Breed	RH and RF vs. SF[1]
Parity	1, 2, ≥3
DIM	1–60, 61–120, 121–180, >180
Time of the year	June–August, September–November, December–February, March–May
Milk yield	Three groups based on 33 and 66 percentiles
Fat[2]	Three groups based on 33 and 66 percentiles
Protein[2]	Three groups based on 33 and 66 percentiles
Urea[2]	Three groups based on 33 and 66 percentiles
Udder hygiene[3]	0–2% dirty vs. 2–10% dirty vs. ≥10% dirty
Udder shape	Nonsymmetric vs. symmetric
Deep udder	Teat tips not below tarsal joint vs. teat tips below tarsal joint
Udder edema	Absent vs. present
Cranial/caudal quarter position	Front quarter vs. rear quarter
Ipsilateral quarter position	Left side vs. right side
Cow IMI status of evaluated CNS species	No other quarters with IMI vs. ≥1 other quarter with IMI caused by same species
Quarter level	
Teat-end callosity roughness[4]	No ring vs. smooth ring vs. rough ring
Teat-end callosity thickness[4]	No ring vs. slight ring vs. moderate + thick ring
Teat shape[4]	Normal vs. not normal[5]
Papilloma at teat tip-region	Absent vs. present
Reddened ring at teat base, after cluster removal	Absent vs. present
Dry teat skin	Absent vs. present
Coinfection with another CNS species	Absent vs. present

[1]RH = Red Holstein; RF = Red Factor; and SF = Swiss Fleckvieh.

[2]According to the closest regular test recording.

[3]According to Winter et al. (2009): 1 = 0 to 2% of udder surface (including perineal area) dirty; 2 = 2 to 10% dirty; 3 = >10% dirty.

[4]According to Neijenhuis et al. (2000).

[5]Not normal: flat, inverted, pointed.

were changed after each cow. After sampling, milking clusters were attached by the respective farmer. Milk samples were transported with a maximum transport time of 40 min at a maximum temperature of 7°C and then frozen at –20°C until further analysis.

Teat and Udder Scorings

Cows' udders were scored before milking. Udder symmetry was categorized binary (symmetric vs. asymmetric). It was also recorded if teat ends were below the tarsal joints. Udder hygiene was scored in 4 categories according to Winter et al. (2009).

Teat end scoring was performed immediately after cluster removal according to Neijenhuis et al. (2000). Teat end callosity roughness was classified as no ring (score 0), smooth (1), or rough (2). Teat end callosity thickness was scored as absent (0), thin (a), moderate (b), thick (c), or extreme (d; Zadoks et al., 2001). Shape of the teat end region was classified as normal (round), flat, cavernous (inverted), or pointed (Neijenhuis et al., 2000). Additionally, papilloma at teat ends were recorded (yes vs. no) and the presence of udder edema was determined visually (yes vs. no). Furthermore, the presence of reddish rings at the teat base due to climbing teat cups during the last phase of the milking process was recorded. Teat end and udder scoring was always performed by 1 of 4 trained observers.

Laboratory Analysis

Milk samples were thawed and centrifuged at 590 × *g* for 10 min at 20°C. Ten microliters of milk sediment was streaked on chromogenic agar plates selective for staphylococci (SaSelect, Bio-Rad, Marnes-la-Coquette, France) and incubated at 37°C for 48 h. Growth of staphylococci from milk on this selective agar was first validated with a collection of 19 different *Staphylococcus* species previously isolated from mastitis milk (Frey et al., 2013). Staphylococci were discriminated from incidentally growing non-staphylococci-like *Corynebacterium* spp., *Bacillus* spp., and *Aerococcus* spp. based on colony shape (round, glossy) and color. *Staphylococcus* isolates were identified at the species level using MALDI-TOF MS (Microflex LT, Bruker Daltonics GmbH, Bremen, Germany) and applying the extraction technique with 70% formic acid and α-cyano-4-hydroxycinnamic acid (HCCA)-matrix (Bruker Daltonics GmbH, Bremen,

Germany). Isolates with scores ≥2.0 were identified to the species level according to manufacturer's guidelines. Each time an isolate displayed a score below 2.0, it was further identified by sequencing of the 16S rRNA gene using universal primers (Kuhnert et al., 1996) as well as by sequencing of the *rpoB* or *dnaJ* genes (Drancourt and Raoult, 2002; Shah et al., 2007). Its spectral profile was introduced into the MALDI-TOF MS database thereafter. Two species with different outcome results than the library stored species in MALDI-TOF MS, and different 16S rRNA and *rpoB* or *dnaJ* sequences were identified as nonvalidated species and were named according to their next closest species based on 16S rRNA identities.

Quarter milk samples with more than 3 different *Staphylococcus* species were not further considered and excluded from the study. Otherwise, up to 3 different *Staphylococcus* species per sample were included.

Definition of IMI

To maximize sensitivity, quarter milk samples were categorized as CNS IMI if ≥100 cfu/mL of the same CNS species from a single sample were identified using the definition with a sensitivity of 80.9% and a specificity of 86.7% for CNS as a group (Dohoo et al., 2011; De Visscher et al., 2016).

Statistical Analyses

Raw data were entered and stored in Excel (Microsoft Excel 2010, Microsoft Corp., Redmond, WA). Data management and statistical analyses were performed using STATA 13 (StataCorp, College Station, TX). The melogit command was applied to build the hierarchical statistical models. The 4 most prevalent CNS species (*Staph. chromogenes*, *Staph. haemolyticus*, *Staph. xylosus*, and the *Staphylococcus warneri*-like species) were analyzed individually. Remaining species, including the 5 other nonvalidated species, were grouped and analyzed as "others." The statistical model for the *Staph. warneri*-like species excluded observations from herds A and B because this species was only found in one sample of herd A and B each.

All statistical models included quarter and cow level random intercepts to correct for clustering of repeated milk samples taken from quarters within cows. The general hierarchical logistic regression model for the probability of CNS IMI was as follows:

$$\text{logit (IMI)} = \text{intercept} + \text{covariates} + u + v + \text{error}, \quad [1]$$

where IMI indicated the presence of a species-specific CNS IMI, and u and v represented the random quarter and cow effects, respectively. Covariates included time of the year, observations on quarters, cows, and bacteriological status of milk samples at the current sample date (Table 1). Covariates on a continuous scale were coded into categories because relationships with CNS species IMI prevalence were not always linear.

Univariable logistic regression models comparing the 5 outcome variables with all potential covariates for CNS IMI were built first. Covariates unconditionally ($P < 0.15$) associated with the presence of CNS IMI were included in further multivariable logistic regression models based on the Wald test. Spearman rank correlation coefficients were calculated among selected variables. If 2 covariates had a correlation coefficient of >0.5, only the biologically more relevant covariate was retained for further analysis to avoid collinearity. Multivariable logistic regression modeling consisted of a stepwise backward elimination process until all covariates were either statistically associated with the outcome variables or a confounder. If coefficients were >0.4, confounding was assumed when coefficients changed more than 25% among nested models. If coefficients were <0.4, confounding was defined if coefficients had changed more than 0.1. Two-way interactions were not evaluated. Statistical significance was Bonferroni-adjusted at P-value <0.01 to account for multiple comparisons between covariates and the 5 most frequent CNS species.

The proportion of variance for species-specific CNS IMI at the quarter and cow levels was estimated based on the final multilevel logistic regression models. Using the latent variable approach, by assuming that the variance at sample level was 3.29 ($\pi^2/3$; Dohoo et al., 2009), the total variance $\left(\sigma^2_{\text{total}}\right)$ was estimated to be

$$\sigma^2_{\text{total}} = \sigma^2_{\text{cow}} + \sigma^2_{\text{quarter}} + \frac{\pi^2}{3},$$

where σ^2_{cow} is the variation occurring at the cow level and $\sigma^2_{\text{quarter}}$ is the variation occurring at the quarter level.

RESULTS

Descriptive Results

Depending on sample date, the 3 herds consisted of 13 to 29 lactating Red Holstein (including Red Factor Black Holstein) and Swiss Fleckvieh cows [herd A: median = 24 (19–27), herd B: median = 17 (13–18),

and herd C: median = 26 (22–29)]. Average 305-d milk yield per herd was 9,182, 7,296, and 7,852 kg for herds A, B, and C, respectively. The arithmetic mean yield corrected bulk milk SCC during the sampling period was 129,000, 97,000, and 155,000 for herd A, B, and C, respectively. Out of 3,422 collected quarter milk samples, 7.9% (n = 271) were not further considered because they contained more than 3 different CNS species, leaving 3,151 monthly quarter observations from 92 cows available for further analysis. Twenty-three different CNS species (Table 2) were identified using MALDI-TOF MS technique, including 2 nonvalidated species. Five quarter milk samples of 5 different cows from herd C had a *Staphylococcus aureus* IMI (≥100 cfu/mL). The most prevalent diagnostic result causing IMI was "others" (11.6%, n = 366), followed by the *Staph. warneri*-like species. This species was identified in 10.2% (n = 320) of total milk samples but was almost exclusively found in herd C (n = 318). *Staph. xylosus* (5.1%, n = 162), *Staph. haemolyticus* (5.1%, n = 160), and *Staph. chromogenes* (3.7%, n = 118) were found less frequently.

Staph. chromogenes

Risk factors associated with *Staph. chromogenes* IMI are presented in Table 3. Udder edema was associated with 206 times higher odds for *Staph. chromogenes* IMI. Odds for *Staph. chromogenes* IMI were lower at the end of the 1-yr longitudinal study (March–May 2015) than during the rest of the study. Quarters of cows belonging to herd B had 109 times higher odds for an IMI with *Staph. chromogenes* compared with quarters from cows of herd C.

Staph. haemolyticus

Risk factors associated with *Staph. haemolyticus* IMI are presented in Table 4. Higher odds for *Staph. haemolyticus* IMI were identified if *Staph. xylosus* or "others" were present within the same quarter at the same sample date with an OR of 3.1 and 3.3, respectively. *Staph. haemolyticus* IMI varied across the study year; odds were the lowest in the third quartile of the study (December 2014–February 2015).

Table 2. Frequencies and percentages of CNS species cultured from 3,422 quarter milk samples in 3 commercial dairy herds (herds A, B, and C) in Switzerland[1]

Species	N[2]	%	Herd A, N (%)	Herd B, N (%)	Herd C, N (%)
Staphylococcus warneri-like[3]	320	10.16	1 (0.03)	1 (0.03)	318 (10.09)
Staphylococcus xylosus	162	5.14	60 (1.71)	69 (2.19)	33 (1.05)
Staphylococcus haemolyticus	160	5.07	75 (2.38)	33 (1.05)	52 (1.65)
Staphylococcus chromogenes	118	3.74	24 (0.8)	75 (2.38)	19 (0.60)
Staphylococcus equorum	86	2.73	46 (1.5)	26 (0.83)	14 (0.44)
Staphylococcus devriesei-like[3]	51	1.62	21 (0.7)	13 (0.41)	17 (0.54)
Staphylococcus auricularis	46	1.46	4 (0.13)	5 (0.16)	37 (1.17)
Staphylococcus sciuri	47	1.49	23 (0.16)	0 (0)	24 (0.76)
Staphylococcus hominis	36	1.14	7 (0.2)	4 (0.13)	25 (0.79)
Staphylococcus saprophyticus	22	0.70	0 (0)	1 (0.03)	21 (0.67)
Staphylococcus devriesei	20	0.63	12 (0.4)	2 (0.06)	6 (0.19)
Staphylococcus vitulinus	14	0.44	1 (0.03)	4 (0.13)	9 (0.29)
Staphylococcus simulans	12	0.38	3 (0.1)	8 (0.25)	1 (0.03)
Staphylococcus epidermidis	9	0.29	5 (0.16)	2 (0.06)	2 (0.06)
Staphylococcus capitis	7	0.22	3 (0.1)	0 (0)	4 (0.13)
Staphylococcus succinus	5	0.16	0 (0)	5 (0.16)	0 (0)
Staphylococcus agnetis[4]	2	0.06	1 (0.03)	0 (0)	1 (0.03)
Staphylococcus cohnii	2	0.06	0 (0)	1 (0.03)	1 (0.03)
Staphylococcus lentus	2	0.06	2 (0.06)	0 (0)	0 (0)
Staphylococcus pasteuri	2	0.06	0 (0)	0 (0)	2 (0.06)
Staphylococcus arlettae	1	0.03	1 (0.03)	0 (0)	0 (0)
Staphylococcus fleuretti	1	0.03	1 (0.03)	0 (0)	0 (0)
Staphylococcus kloosii	1	0.03	0 (0)	0 (0)	1 (0.03)
Staphylococcus aureus	5	0.16	0 (0)	0 (0)	5 (0.16)
>3 CNS species	271	7.92	51 (4.4)	74 (9.5)	146 (12.1)
Noninfected quarters	3,151		1,163	779	1,209
Total number of quarters	3,422		1,214	853	1,355

[1]More than one species per sample was allowed.

[2]Number of positive (≥100 cfu/mL) milk samples.

[3]Nonvalidated species.

[4]Coagulase variable.

Table 3. Final multilevel multivariable logistic regression model of risk factors associated with quarter-level *Staphylococcus chromogenes* IMI in 90 cows in 3 Swiss dairy herds (n = 2,809)[1]

Variable	Category	Frequency	Prevalence	Odds ratio	95% CI	*P*-value
Herd	A	1,051	17 (1.6%)	5.5	0.4–78.2	0.207
	B	747	71 (9.5%)	109.8	6.1–1,967.5	0.001
	C	1,011	16 (1.6%)	Ref.		
Time of year	June–August	699	36 (5.2%)	6.0	2.1–16.8	0.001
	September–November	625	27 (4.3%)	4.4	1.5–12.7	0.087
	December–February	624	25 (4.0%)	3.4	1.2–9.4	0.001
	March–May	861	16 (1.9%)	Ref.		
Edema	Yes	34	10 (29.4%)	206.4	21.8–1,956.1	<0.001
	No	2,775	94 (3.4%)	Ref.		

[1]Variance of random quarter and cow effect was 14.77 and 6.33, respectively. Ref. = referent.

Staph. warneri-Like Species

The *Staph. warneri*-like species could not be properly identified to the species level based on MALDI-TOF identification, 16S rRNA sequence, and *rpoB* and *dnaJ* sequences. This species showed the closest identity to *Staph. warneri* with 99% 16S rRNA identity, 97% *rpoB* identity, and 94% *dnaJ* identity and was therefore not considered as a true *Staph. warneri*. The models for the *Staph. warneri*-like species were only based on the isolates of farm C because only 2 *Staph. warneri* isolates were collected on the other farms.

Risk factors associated with *Staph. warneri*-like species IMI are presented in Table 5. Prevalence of *Staph. warneri*-like IMI increased in the first half of the study, whereas it decreased again in the second half. The concurrent presence of *Staph. xylosus* within the same quarter increased the odds of *Staph. warneri*-like IMI 9.2 times. Cows after peak production (>60 DIM) had higher odds for *Staph. warneri*-like IMI compared with cows within the first 60 DIM.

Staph. xylosus

Risk factors associated with *Staph. xylosus* IMI are presented in Table 6. Odds for *Staph. xylosus* IMI were the highest in early lactation (DIM <60 d) and the first 3 mo of the study (June to August 2014). Quarters with a *Staph. haemolyticus* (OR = 3.5) or an "others" coinfection (OR = 2.6) had higher odds to be infected with *Staph. xylosus* IMI. Prevalence of *Staph. xylosus* IMI was the highest in herd B and the lowest in herd C.

Others

Risk factors associated with "others" IMI are presented in Table 7. The presence of *Staph. haemolyticus* IMI (OR = 3.1) or *Staph. xylosus* (OR = 2.1) in the same quarter was significantly associated with "others" IMI.

Variance Components

Variance components of all final models are presented in Table 8 and differed between the individual species. Variance estimates on individual sample level were the highest for the species *Staph. haemolyticus*, *Staph. warneri*-like, *Staph. xylosus*, and "others." Estimates were highest for *Staph. chromogenes* on the quarter level. A variance estimate on cow level >20% was found for *Staph. chromogenes* IMI and *Staph. warneri*-like IMI and were lower for the other species.

Table 4. Final multilevel multivariable logistic regression model of risk factors associated with quarter-level *Staphylococcus haemolyticus* IMI in 91 cows in 3 Swiss dairy herds (n = 2,766)[1]

Variable	Category	Frequency	Prevalence	Odds ratio	95% CI	*P*-value
Time of year	June–August	609	45 (7.4%)	3.4	1.8–6.6	<0.001
	September–November	611	32 (5.2%)	2.8	1.4–5.5	0.004
	December–February	649	14 (2.2%)	Ref.		
	March–May	897	36 (4.0%)	2.1	1.1–4.1	0.030
Coinfection with *Staphylococcus xylosus*	Yes	135	15 (11.1%)	3.1	1.5–6.3	0.002
	No	2,631	112 (4.3%)	Ref.		
Coinfection with "others"[2]	Yes	297	33 (11.1%)	3.3	2.0–5.4	<0.001
	No	2,469	94 (3.8%)	Ref.		

[1]Variance of random quarter and cow effect was 1.10 and 0.73, respectively. Ref. = referent.

[2]Coagulase-negative staphylococci IMI caused by CNS species other than *Staphylococcus chromogenes*, *Staph. haemolyticus*, *Staphylococcus warneri*-like, and *Staph. xylosus*.

Table 5. Final multilevel multivariable logistic regression model of risk factors associated with quarter-level *Staphylococcus warneri*-like IMI in 35 cows in 1 Swiss dairy herd (n = 1,007)[1]

Variable	Category	Frequency	Prevalence	Odds ratio	95% CI	*P*-value
DIM	>180	461	144 (31.2%)	6.9	3.6–13.2	<0.001
	121–180	181	49 (27.1%)	5.9	2.9–12.3	<0.001
	61–120	171	49 (28.7%)	5.3	2.6–11.1	<0.001
	1–60	194	19 (9.8%)	Ref.		
Time of year	June–August	264	51 (19.3%)	Ref.		
	September–November	229	73 (31.9%)	2.5	1.4–4.3	0.001
	December–February	246	71 (28.9%)	2.6	1.5–4.6	0.001
	March–May	268	66 (24.6%)	1.9	1.1–3.2	0.029
Coinfection with *Staphylococcus xylosus*	Yes	23	10 (43.5%)	9.2	2.6–31.9	<0.001
	No	984	251 (25.5%)	Ref.		

[1]Variance of random quarter and cow effect was 2.13 and 1.44, respectively. Ref. = referent.

DISCUSSION

In this longitudinal study, *Staph. chromogenes*, *Staph. haemolyticus*, *Staph. xylosus*, and a *Staph. warneri*-like species were the most prevalent CNS species. Except for *Staph. warneri*-like species, this is in agreement with other European studies on CNS IMI (Supré et al., 2011; Frey et al., 2013). It must be noted that the *Staph. warneri*-like species described in our study may represent a novel *Staphylococcus* species based on preliminary sequence data. Other frequently observed species in previous European studies were *Staphylococcus epidermidis*, *Staph. simulans*, or *Staphylococcus cohnii* (Sampimon et al., 2009; Thorberg et al., 2009), but those were rarely seen in the 3 herds of our study. In a recent review, *Staph. chromogenes*, *Staph. haemolyticus*, *Staph. epidermidis*, *Staph. simulans*, and *Staph. xylosus* were considered the most important CNS species causing IMI (Vanderhaeghen et al., 2015), but patterns of CNS species are frequently herd specific (Piessens et al., 2011; Supré et al., 2011). Within our study herds, a herd-specific pattern was particularly pronounced for the *Staph. warneri*-like species, which was mainly identified in samples from farm C. The other important species were isolated on all 3 farms but *Staph. chromogenes* was predominant in herd B. The predominance of *Staph. chromogenes* in 1 of our study herds and the isolation of *Staph. haemolyticus* and *Staph. xylosus* in all 3 herds corresponded with findings of another study showing that *Staph. chromogenes* was mainly present in milk samples, whereas *Staph. haemolyticus* was more often identified in samples collected from the cow's environment (Piessens et al., 2011), partly explaining herd specific species distribution. However, only 3 herds were included in the present study and therefore results need to be interpreted and compared with care.

Recently, Souza et al. (2016) showed a different in vitro behavior of various CNS strains (*Staphylococcus*

Table 6. Final multilevel multivariable logistic regression model of risk factors associated with quarter-level *Staphylococcus xylosus* IMI in 90 cows in 3 Swiss dairy herds (n = 2,809)[1]

Variable	Category	Frequency	Prevalence	Odds ratio	95% CI	*P*-value
Herd	A	1,051	46 (4.4%)	2.9	1.2–6.9	0.017
	B	747	66 (8.8%)	6.8	2.7–16.9	<0.001
	C	1,011	19 (1.9%)	Ref.		
DIM	>180	1,284	51 (4.0%)	Ref.		
	121–180	519	20 (3.9%)	1.0	0.5–1.9	0.987
	61–120	524	27 (5.2%)	1.4	0.8–2.6	0.256
	1–60	482	33 (6.9%)	2.6	1.5–4.5	0.001
Time of year	June–August	699	64 (9.2%)	6.5	3.5–12.0	<0.001
	September–November	625	18 (2.9%)	1.2	0.6–2.5	0.595
	December–February	624	28 (4.5%)	2.0	1.0–3.9	0.044
	March–May	861	21 (2.4%)	Ref.		
Coinfection with "others"[2]	Yes	296	25 (8.5%)	2.6	1.5–4.6	0.001
	No	2,513	106 (4.2%)	Ref.		
Coinfection with *Staphylococcus haemolyticus*	Yes	137	16 (11.7%)	3.5	1.7–7.4	0.001
	No	2,672	115 (4.3%)	Ref.		

[1]Variance of random quarter and cow effect was 1.41 and 0.86, respectively. Ref. = referent.

[2]Coagulase-negative staphylococci IMI caused by CNS species other than *Staphylococcus chromogenes*, *Staph. haemolyticus*, *Staphylococcus warneri*-like, and *Staph. xylosus*.

Table 7. Final multilevel multivariable logistic regression model of risk factors associated with quarter-level "others"[1] IMI in 90 cows in 3 Swiss dairy herds (n = 2,780)[2]

Variable	Category	Frequency	Prevalence	Odds ratio	95% CI	*P*-value
Coinfection with *Staphylococcus xylosus*	Yes	131	25 (19.1%)	2.1	1.3–3.4	0.004
	No	2,649	266 (10.0%)	Ref.		
Coinfection with *Staphylococcus haemolyticus*	Yes	134	33 (24.6%)	3.1	2.0–4.8	<0.001
	No	2,646	258 (9.8%)	Ref.		

[1]Coagulase-negative staphylococci IMI caused by CNS species other than *Staphylococcus chromogenes*, *Staph. haemolyticus*, *Staphylococcus warneri*-like, and *Staph. xylosus*.

[2]Variance of random quarter and cow effect was 0.21 and 0.14, respectively. Ref. = referent.

fleuretti and 2 different strains of *Staph. chromogenes*) when they interacted with bovine mammary epithelial cells. *Staphylococcus chromogenes* cultured from a chronic IMI showed greater ability to adhere and internalize into bovine mammary epithelial cells than *Staph. chromogenes* originating from an extra-mammary sample. All investigated CNS species adhered slower and showed a slower internalization than *Staph. aureus* (Souza et al., 2016). The particular ability of *Staph. chromogenes* isolated from milk to possess some degree of host adaptation might explain at least partly the predominance of this species in certain herds including study herd B.

An important risk factor identified in all models, except for "others," was the time of the year. For *Staph. chromogenes*, *Staph. haemolyticus*, and *Staph. xylosus*, the months June to November and June to August, respectively, were associated with higher odds for IMI, similar to the results of De Visscher et al. (2017) who found an increased risk for presence of more relevant species in bulk tank milk in June and September. Because both species are frequently found in the cow's environment (Matos et al., 1991; Piessens et al., 2011), these associations might be caused by a high environmental bacterial pressure caused by warm weather conditions in summer and early autumn. In contrast, the months from September to February were associated with higher odds for *Staph. warneri*-like IMI in herd C. Little information is available on *Staph. warneri* because it was rarely reported in former studies (Sampimon et al., 2009; Thorberg et al., 2009; Piessens et al., 2011). However, Mørk et al. (2012) estimated a restricted survival period of *Staph. warneri* within the bovine udder. The association with the time of the year found in the present study therefore might be attributable to an increased environmental pressure during the indoor period or to a contagious behavior of the *Staph. warneri*-like species present in herd C. The latter may be supported by the fact that a higher mean number of DIM of the lactating cows increased the odds for *Staph. warneri*-like IMI. Additionally, herd data revealed (data not shown) that during the winter months the mean number of DIM of all lactating cows in herd C was higher compared with the other periods of the study year. Additional strain typing or the calculation of transmission parameters is needed to confirm this hypothesis.

In a Belgian study, De Visscher et al. (2015) found that heifers and cows in early lactation (<60 DIM) were more often infected with the more relevant CNS species (*Staph. chromogenes*, *Staph. simulans,* and *Staph. xylosus*). Parity was not significantly associated with CNS IMI in our study. However, DIM was a significant risk factor in the final models for *Staph. xylosus* (highest risk for cows <60 DIM) and *Staph. warneri*-like species (highest risk for cows >120 DIM). This confirms the

Table 8. Variance component estimation at the cow, quarter, and sample level for all 5 final multilevel logistic regression models (*Staphylococcus chromogenes*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*-like, *Staphylococcus xylosus*, and "others")[1] of 90 cows in 3 Swiss dairy herds

	Staph. chromogenes		*Staph. haemolyticus*		*Staph. warneri*-like		*Staph. xylosus*		"Others"	
Item	Var. est.[2]	%	Var. est.	%	Var. est.	%	Var. est.	%	Var. est.	%
Cow	6.72	27.3	0.12	2.4	0	0	0.14	2.7	0.04	1.1
Quarter	14.61	59.3	1.60	31.9	3.50	51.5	1.70	33.1	0.39	10.5
Sample	3.29	13.4	3.29	65.7	3.29	48.5	3.29	64.1	3.29	88.4
Total	24.62	100	5.01	100	6.79	100	5.13	100	3.72	100

[1]Coagulase-negative staphylococci IMI caused by CNS species other than *Staph. chromogenes*, *Staph. haemolyticus*, *Staph. warneri*-like, and *Staph. xylosus*.

[2]Variance estimation.

finding that the risk for IMI with more relevant CNS species such as *Staph. chromogenes*, *Staph. xylosus*, and *Staphylococcus simulans* is higher in early lactation (De Visscher et al., 2015). Additionally, udder edema was a significant cow level risk factor for *Staph. chromogenes* IMI in the present study. Udder edema was shown in earlier studies to increase the risk for IMI caused by other pathogens in dairy heifers (Piepers et al., 2011; Krömker et al., 2012). Waage et al. (2001) assumed a decreased blood circulation and a poor adaptation to the milking units in teats with edema leading to a decrease in udder defense mechanisms to be the underlying cause of this association.

No classical quarter level covariates (i.e., teat end score, papillomatosis, teat skin lacerations, and so on) were identified to be significant risk factors for species-specific CNS IMI in the present study. This is similar to the results of a German study in which no significant associations between teat end condition and new IMI caused by CNS or major mastitis pathogens were identified (Zoche-Golob et al., 2015). In contrast, associations of high teat end scores with a high infection rate of *Staph. aureus* (Zadoks et al., 2001), high loads of *Streptococcus uberis* and *Escherichia coli* in the teat canals (Paduch et al., 2012; Zoche-Golob et al., 2015), and occurrence of clinical mastitis (Neijenhuis et al., 2001) were described by others. Recently, De Visscher et al. (2016) found an increased risk for IMI with *Staph*, *chromogenes*, *Staph. simulans*, and *Staph. xylosus* for quarters with a protuberant or inverted teat end score. Unfortunately, it is difficult to compare these studies in detail because different IMI definitions were used and different bacteria were investigated. However, associations with mastitis occurrence were found only in cases with extreme hyperkeratosis and inverted teat ends. In the present study, no quarters with extreme thick rings (2d) according to the definitions of Neijenhuis et al. (2000) were found. Only 66 out of 3,422 teat end observations were scored with rough and thick rings (2b). The lack of extreme hyperkeratosis in our study and the repeated measurements in a relatively small number of cows might explain that no significant association was identified between teat end condition and CNS IMI.

Coinfections with other CNS species within the same quarter were relevant in the final models for *Staph. xylosus*, *Staph. haemolyticus*, *Staph. warneri*-like species, and "others." To our knowledge, no detailed research on CNS coinfections has been published to date. What was defined as coinfection might happen to be a sample positive for 2 different species due to teat apex or teat canal colonization. A recent study by De Visscher et al. (2016) found an association between *Staph. chromogenes* IMI and teat apex colonization with other species as well as with the same species in fresh cows, but no information is available for *Staph. haemolyticus* and *Staph. xylosus*, which are both prevalent in the environment (Piessens et al., 2011). Potential causes for a positive association might be a combination of distinct virulence factors, synergism in bacteria metabolism, and environmental conditions such as poor hygiene, but the underlying mechanisms remain unclear.

The individual cow's immune status as a potential risk factor was not assessed but might be relevant for species such as *Staph. chromogenes* and the *Staph. warneri*-like species where 26 and 21% of the variance, respectively, were attributable to the cow level. This corresponds well with the fact that *Staph. chromogenes* is a cow-associated mastitis pathogen with the udder as its reservoir but causes infection in an opportunistic way because it was found on teat and udder skin as well as in milk samples (Vanderhaeghen et al., 2015). A contagious behavior cannot be excluded because identical strains were found in several animals of one herd in other studies (Taponen et al., 2008; Mørk et al., 2012). The biggest variance occurred on the sample level. This might be explained by temporal changes in CNS prevalence caused by the self-cure rate of pathogens, or hygiene and management problems in the herds. Investigating temporal changes was beyond the aim of this study.

CONCLUSIONS

Risk factors for species-specific CNS IMI were identified in this longitudinal study. The summer and early autumn months were associated with a *Staph. haemolyticus*, *Staph. chromogenes*, and *Staph. xylosus* IMI, whereas the risk for *Staph. warneri*-like species was increased during the indoor period. Coinfections were identified for *Staph. haemolyticus*, *Staph. xylosus*, *Staph. warneri*-like, and "others" IMI, although their role needs to be further investigated. The presence of herd-dependent CNS species distributions emphasizes the importance of an accurate identification of isolates at the species level.

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REFERENCES

De Visscher, A., S. Piepers, F. Haesebrouck, and S. De Vliegher. 2016. Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health. J. Dairy Sci. 99:6457–6469. https://doi.org/10.3168/jds.2015-10458.

De Visscher, A., S. Piepers, F. Haesebrouck, K. Supré, and S. De Vliegher. 2017. Coagulase-negative Staphylococcus species in bulk milk: Prevalence, distribution, and associated subgroup- and species-specific risk factors. J. Dairy Sci. 100:629–642. https://doi.org/10.3168/jds.2016-11476.

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. https://doi.org/10.3168/jds.2014-9088.

Dohoo, I., W. Martin, and H. Stryhn. 2009. Veterinary Epidemiologic Research. 2nd ed. VER Inc., Charlottetown, Prince Edward Island, Canada.

Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J. Dairy Sci. 94:250–261. https://doi.org/10.3168/jds.2010-3559.

Drancourt, M., and D. Raoult. 2002. rpoB gene sequence-based identification of *Staphylococcus* species. J. Clin. Microbiol. 40:1333–1338. https://doi.org/10.1128/JCM.40.4.1333.

Dufour, S., I. R. Dohoo, H. W. Barkema, L. Descôteaux, T. J. Devries, K. K. Reyher, J.-P. Roy, and D. T. Scholl. 2012. Epidemiology of coagulase-negative staphylococci intramammary infection in dairy cattle and the effect of bacteriological culture misclassification. J. Dairy Sci. 95:3110–3124. https://doi.org/10.3168/jds.2011-5164.

Frey, Y., J. P. Rodriguez, A. Thomann, S. Schwendener, and V. Perreten. 2013. Genetic characterization of antimicrobial resistance in coagulase-negative staphylococci from bovine mastitis milk. J. Dairy Sci. 96:2247–2257. https://doi.org/10.3168/jds.2012-6091.

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. https://doi.org/10.3168/jds.2013-7657.

Krömker, V., F. Pfannenschmidt, K. Helmke, R. Andersson, and N. T. Grabowski. 2012. Risk factors for intramammary infections and subclinical mastitis in post-partum dairy heifers. J. Dairy Res. 79:304–309. https://doi.org/10.1017/S0022029912000222.

Kuhnert, P., S. E. Capaul, J. Nicolet, and J. Frey. 1996. Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences. Int. J. Syst. Bacteriol. 46:1174–1176. http://dx.doi.org/https://doi.org/10.1099/00207713-46-4-1174.

Matos, J. S., D. G. White, R. J. Harmon, and B. E. Langlois. 1991. Isolation of *Staphylococcus aureus* from sites other than the lactating mammary gland. J. Dairy Sci. 74:1544–1549. http://dx.doi.org/https://doi.org/10.3168/jds.S0022-0302(91)78315-X.

Matthews, K. R., R. J. Harmon, and B. E. Langlois. 1992. Prevalence of *Staphylococcus* species during the periparturient period in primiparous and multiparous cows. J. Dairy Sci. 75:1835–1839. https://doi.org/10.3168/jds.S0022-0302(92)77942-9.

Mørk, T., H. J. Jørgensen, M. Sunde, B. Kvitle, S. Sviland, S. Waage, and T. Tollersrud. 2012. Persistence of staphylococcal species and genotypes in the bovine udder. Vet. Microbiol. 159:171–180. https://doi.org/10.1016/j.vetmic.2012.03.034.

Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. Noordhuizen. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. J. Dairy Sci. 83:2795–2804. https://doi.org/10.3168/jds.S0022-0302(00)75177-0.

Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. P. Noordhuizen. 2001. Relationship between teat-end callosity and occurrence of clinical mastitis. J. Dairy Sci. 84:2664–2672.

Paduch, J.-H., E. Mohr, and V. Krömker. 2012. The association between teat end hyperkeratosis and teat canal microbial load in lactating dairy cattle. Vet. Microbiol. 158:353–359.

Piepers, S., L. De Meulemeester, A. de Kruif, G. Opsomer, H. W. Barkema, and S. De Vliegher. 2007. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. J. Dairy Res. 74:478–483. https://doi.org/10.1017/S0022029907002841.

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. https://doi.org/10.1016/j.prevetmed.2011.02.010.

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 milk and environment of dairy cows differs between herds. J. Dairy Sci. 94:2933–2944. https://doi.org/10.3168/jds.2010-3956.

Roesch, M., M. G. Doherr, W. Schären, M. Schällibaum, and J. W. Blum. 2007. Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. J. Dairy Res. 74:86–92. https://doi.org/10.1017/S002202990600210X.

Rüegsegger, F., J. Ruf, A. Tschuor, Y. Sigrist, M. Rosskopf, and M. Hässig. 2014. Antimicrobial susceptibility of mastitis pathogens of dairy cows in Switzerland. Schweiz. Arch. Tierheilkd. 156:483–488. https://doi.org/10.1024/0036-7281/a000635.

Sampimon, O. C., H. W. Barkema, I. M. Berends, J. Sol, and T. J. Lam. 2009. Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet. Microbiol. 134:37–44. https://doi.org/10.1016/j.vetmic.2008.09.010.

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. https://doi.org/10.1016/j.vetmic.2008.09.014.

Shah, M. M., H. Iihara, M. Noda, S. X. Song, P. H. Nhung, K. Ohkusu, Y. Kawamura, and T. Ezaki. 2007. dnaJ gene sequence-based assay for species identification and phylogenetic grouping in the genus *Staphylococcus*. Int. J. Syst. Evol. Microbiol. 57:25–30. https://doi.org/10.1099/ijs.0.64205-0.

Souza, F. N., S. Piepers, A. M. M. P. Della Libera, M. B. Heinemann, M. M. O. P. Cerqueira, and S. De Vliegher. 2016. Interaction between bovine-associated coagulase-negative staphylococci species and strains and bovine mammary epithelial cells reflects differences in ecology and epidemiological behavior. J. Dairy Sci. 99:2867–2874. https://doi.org/10.3168/jds.2015-10230.

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. https://doi.org/10.3168/jds.2010-3741.

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. https://doi.org/10.1017/S0022029908003312.

Tenhagen, B. A., G. Köster, J. Wallmann, and W. Heuwieser. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci. 89:2542–2551. https://doi.org/10.3168/jds.S0022-0302(06)72330-X.

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. https://doi.org/10.3168/jds.2009-2184.

Tomazi, T., J. L. Gonçalves, J. R. Barreiro, M. a. Arcari, and M. V. dos Santos. 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. J. Dairy Sci. 98:3071–3078. https://doi.org/10.3168/jds.2014-8466.

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. https://doi.org/10.1016/j.tvjl.2014.11.001.

Waage, S., S. A. Ødegaard, A. Lund, S. Brattgjerd, and T. Røthe. 2001. Case-control study of risk factors for clinical mastitis in postpartum dairy heifers. J. Dairy Sci. 84:392–399. https://doi.org/10.3168/jds.S0022-0302(01)74489-X.

Winter, P., C. Burvenich, H. Hogeveen, F. Neijenhuis, and B. de Spiegeleer. 2009. Praktischer Leitfaden Mastititstle. P. Winter, ed. Parey Verlag, Vienna, Austria.

Zadoks, R. N., H. G. Allore, H. W. Barkema, O. C. Sampimon, G. J. Wellenberg, Y. T. Gröhn, and Y. H. Schukkent. 2001. Cow- and quarter-level risk factors for *Streptococcus uberis* and *Staphylococcus aureus* mastitis. J. Dairy Sci. 84:2649–2663. https://doi.org/10.3168/jds.S0022-0302(01)74512-2.

Zoche-Golob, V., H. Haverkamp, J.-H. Paduch, D. Klocke, C. Zinke, M. Hoedemaker, W. Heuwieser, and V. Krömker. 2015. Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows. J. Dairy Sci. 98:910–917. https://doi.org/10.3168/jds.2014-8446.