***Interpretive summary***

Variation in distribution and diversity of *Staphylococcus* species causing intramammary infections in dairy cattle is associated with different management practices. The objective of the current study was to identify which *Staph*. species are most relevant to udder health for organic dairies, by exploring how quarter somatic cell count (SCC) varied as a result of infection caused by the most frequently isolated species. Compared to healthy quarters, SCC was higher in quarters infected with 9 of 10 *Staph.* species. Although the increase in SCC was modest for most species observed, their widespread nature can still result in increased bulk tank SCC.

***Running head:***

Staphylococci mastitis on organic dairy farms

***Title***

Staphylococci and mammaliicocci: which species are important for udder health on organic dairy farms?

C. E. Jeffrey,1 P. R. F. Adkins,2 S. Dufour,3 and J. W. Barlow1

1 Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT 05405

2 Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO 65211

3 Faculty of veterinary medicine, Université de Montréal, St-Hyacinthe, QC, Canada

Corresponding author: John Barlow

Department of Animal and Veterinary Sciences,

202 Terrill Building,

University of Vermont,

Burlington, VT 05405

Phone: 802-656-1395

Email: [john.barlow@uvm.edu](mailto:john.barlow@uvm.edu)

***Abstract***

Variation in species distribution and diversity of staphylococci and mammaliicocci (SaM) species causing intramammary infections in dairy cattle is associated with different management practices. Disparate selective pressures on organic dairies could potentially result in population differences of these mastitis-causing bacteria. No previous studies have explored the species-specific effect on quarter somatic cell count of SaM for a population of certified organic dairies. The current study presents data from a longitudinal, cross-sectional study of 10 certified organic dairy farms. The objective was to estimate how quarter somatic cell count (qSCC) varied as a result of infection with the most frequently isolated SaM species. Aerobic culture of quarter-milk samples to identify IMI was conducted in parallel with determination of qSCC. A linear hierarchical repeated measures mixed model was used to estimate qSCC for quarters with an IMI caused by a given SaM species, compared to healthy (no growth) quarters. The model included days in milk at time of sampling to adjust qSCC estimates for each SaM species. The final data set consisted of 648 quarters with an IMI due to 10 different SaMspp. and 1,972 healthy quarters. *S. chromogenes* was the most frequent species, followed by *S.* *aureus, S. haemolyticus,* and *S.* *simulans.* A large amount of variability was observed in the somatic cell score for healthy quarters and those infected with many SaM spp., especially *S. chromogenes, S. haemolyticus, S. simulans,* and *S. aureus.* Somatic cell score was significantly higher in quarters infected with *S. agnetis, S. aureus, S. chromogenes, S. devriesei, S. haemolyticus, S. hyicus, S. simulans, S. warneri, and S. xylosus* compared to healthy quarters. The highest cell count was for quarters infected with *S. warneri,* followed by *S.* *aureus, S. agnetis,* and *S. hyicus.* The relative distribution of various SaMspecies and their effect on qSCC in this population of small to midsize organic farms was similar to previous studies describing conventionally-managed dairies. Although the increase in qSCC was modest for most SaM species observed, the widespread nature of these intramammary pathogens can still result in sizeable increases in bulk tank SCC.

***Keywords:***

Mastitis, organic dairy cattle, staphylococci and mammaliicocci, quarter-level somatic cell count, intramammary infection

***Introduction***

Staphylococci and mammaliicocci are the predominant pathogens causing intramammary infections in dairy animals globally. This group includes the major mastitis pathogen *Staph. aureus*, and a heterogeneous group of bacteria known as the non-*aureus* staphylococci and mammaliicocci (SaM). For many dairy farms that have implemented modern mastitis control practices minimizing the effects of “major” pathogens such as *Staph. aureus*, the leading contributor to bulk tank milk SCC on farms with good milk quality is IMI due to other species within the SaM (Schukken et al., 2009). SaM cow-level prevalence in one US study was 71% (Jenkins et al., 2019), and a quarter-level prevalence of 11, 26, 21, and 33% has been reported in the US, Canada, and two Belgian studies, respectively (Condas et al., 2017a; Rowe et al., 2019; Wuytack et al., 2020; Valckenier et al., 2021). Although primarily associated with cases of subclinical mastitis (Persson Waller et al., 2011; Heikkilä et al., 2018), SaM are also capable of causing clinical mastitis (Taponen et al., 2007; Simojoki et al., 2009; Verbeke et al., 2014; Condas et al., 2017b; Wuytack et al., 2020). Taken as a group, SaM IMI likely have minimal detrimental effect on milk yield (Tomazi et al., 2015; Valckenier et al., 2020) and can have a high rate of spontaneous cure (Taponen et al., 2007; Valckenier et al., 2020), but many SaM species have been shown to increase somatic cell count (Supré et al., 2011; Tomazi et al., 2015; Condas et al., 2017b; Valckenier et al., 2019), as well as persist for long periods of time in the udder (Piessens et al., 2011; Nyman et al., 2018; Valckenier et al., 2021).

SaM are an incredibly heterogenous group of bacteria, with studies identifying at least 25 different species as causing IMI in dairy cattle (Condas et al., 2017a; De Visscher et al., 2017). Different SaM species vary widely in both their epidemiology and ecology; some are considered primarily host-adapted (colonizing the skin or udder), while others are primarily found in the cow’s environment (as reviewed in De Buck et al., 2021). Certain species have been associated with stall surfaces, air, and unused sawdust bedding material (Piessens et al., 2011), some with different facility types (Condas et al., 2017a), and others with environmental contamination and poor teat hygiene at milking time (De Visscher et al., 2016; De Visscher et al., 2017). SaM species also differ in how they behave as intramammary pathogens; the ability to cause persistent infections varies by species (Nyman et al., 2018; Valckenier et al., 2021), as well as the presence of antimicrobial resistance determinants (Frey et al., 2013; Fergestad et al., 2021), virulence potential (Naushad et al., 2019; França et al., 2021), and interaction with a host’s immune system (Åvall-Jääskeläinen et al., 2013; Breyne et al., 2015).

Perhaps most importantly for the overall udder health status of a dairy farm as measured by bulk tank SCC, SaM species also vary in the degree to which they cause an inflammatory reaction in the udder (Supré et al., 2011; Nyman et al., 2018; Wuytack et al., 2020; Taponen et al., 2022). However, only a limited number of studies have described the effect of the breadth of observed species on quarter-level SCC using observations from multiple herds, where isolates were identified using MALDI-TOF or genotypic methods, and accounting for days in milk at time of observation (Fry et al., 2014; Condas et al., 2017b). Although infection status is the most important factor, stage of lactation has a significant effect on SCC (Schutz et al., 1990; Schepers et al., 1997). No previous studies have identified which *Staph.* species are most relevant to udder health by describing the species-specific effect on SCC for a population of certified organic dairies. Although similar in many general aspects, organic and conventional dairies differ significantly in a number of ways both in management (Stiglbauer et al., 2013), and treatments and attitudes around mastitis (Ruegg, 2009). For example, in the absence of antibiotic use on organic dairies, antimicrobial susceptibility of common mastitis pathogens can differ between conventional and organic dairy farms in the US (Tikofsky et al., 2003; Pol and Ruegg, 2007; Bombyk et al., 2008). Given that variation in SaM species distribution and diversity is associated with a variety of different management practices (Dufour et al., 2012; Condas et al., 2017a), it is possible that these differences may create disparate selective pressure between conventional and organic farms, potentially resulting in differences in virulence and impact on SCC.

The current study presents data from a longitudinal, cross-sectional study of 10 certified organic dairy farms in Vermont, US. Microbiological analyses of quarter-milk samples to identify IMI due to staphylococci and mammaliicocci were conducted in parallel with determination of quarter-level somatic cell count. The objective of this study was to estimate how quarter-milk SCC varied as a result of infection with the most frequently isolated *Staph.* species, in order to identify which species were more relevant to udder health in this population of farms.

***Materials and methods***

*Sample origination*

Samples included in the current study were collected during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US) carried out in Winter 2019-2020. Enrolled farms were a non-probability subsample of certified organic dairies in Vermont which had participated in previous studies, and inclusion criteria included: 1) milking between 35-120 cows and 2) using either a tiestall barn bedded with shavings/sawdust or a bedded pack system to house lactating dairy cows. For the purposes of a separate study, an equal number of herds using each of the two bedding types were enrolled. Five farms housed cows in a tiestall bedded with wood shavings, and 5 utilized a bedded pack system (3 actively managed for composting, 2 static). Around the time of the first farm visit, herd records were captured from the record processing center working with 9 of the participating herds (Lancaster DHIA, Manheim, PA; Dairy One Co-Op. Inc., Ithaca, NY) to obtain freshening date and parity for the current lactation. Freshening date and parity for 1 herd was obtained from personal communication with the producer who kept written records. The goal was to enroll 35 cows of varying parity in early- to mid-lactation from each herd for the duration of the study. In 1 herd with approximately 35 lactating cows, all cows were sampled. In 8 herds with ≥ 35 cows and with available DHIA data, a stratified random approach was used with cows stratified by SCC, lactation number, and DIM and then randomly selected across these variables. In 1 herd with ≥ 35 cows and no DHIA data, the producer generated a list of 35 cows in early lactation so that they would continue to be milking for the duration of the study. Cows that were unable to be sampled at a follow-up visit (dried off, left the herd) were replaced with another lactating cow dictated by convenience. At each farm visit, duplicate quarter-milk samples were aseptically collected from each lactating quarter immediately before milking for all enrolled cows according to NMC guidelines (NMC, 2017). Briefly, after routine pre-milking teat disinfection was completed, researchers (wearing clean disposable gloves) scrubbed teat ends and the distal third of teats with 70% isopropyl alcohol-moistened gauze swabs until teat ends were visibly clean, stripped the quarters (discarding 3-5 squirts of foremilk), and sequentially collected approximately 5-6 mL of milk into each of two sterile 11-mL flip-top vials. Samples were kept on ice in a cooler during transport until stored temporarily overnight at 4°C in the laboratory, where an aliquot was frozen for SCC measurement and the remaining milk sample was processed for bacteriological culture.

*SCC measurement*

Aliquots of frozen quarter-milk samples were sent to the Vermont State Agricultural and Environmental Laboratory, where samples were thawed at time of processing and quarter-level somatic cell count was determined using flow cytometry (Somacount FC, Bentley Instruments).

*Aerobic culture of milk samples*

Standard aerobic bacteriological culture of quarter-milk was performed in duplicate within 24 hours of collection to identify bacterial species present in the sample. the IMI status of each quarter. After being homogenized by gentle inversion, tryptic soy agar plates with 5% sheep blood (Northeast Laboratory, Waterville, ME) were inoculated with 10 μL of milk using disposable plastic inoculating loops. Plates were then incubated in aerobic conditions at 37°C before being read at approximately 48 hrs.

The results of aerobic culture of the duplicate samples was used to determine the overall status of each quarter-milk sample: 1) “no growth,” when there was no significant growth on either plate (i.e., no growth on both plates, or ≤ 200 CFU/mL on one plate and no growth on the other plate, or ≤ 200 CFU/mL on both plates and morphology of isolates on each plate was different; 2) “pure culture,” when there was ≥ 100 CFU/mL of a particular isolate identified with the same morphology on both plates; 3) “mixed culture,” when there was ≥ 100 CFU/mL of two phenotypically-distinct isolates identified, each growing on both plates; and 4) “contaminated,” when ≥ 1 of the 2 samples had more than 2 morphologically distinct isolates growing on a plate.

A quarter was considered positive for an IMI when ≥ 100 CFU/mL of a particular isolate was identified with the same morphology on both plates (interpretation in series; Dohoo et al., 2011). A quarter was considered negative or healthy/no growth when there was no significant growth on either plate (i.e., no growth on both plates, or no significant growth on one plate and no growth on the other plate, or no significant growth on both plates and morphology of isolates on each plate was different). Quarter-milk samples were classified as contaminated if more than 2 different morphologically distinct isolates grew on a plate. If ≥ 1 of the 2 samples were classified as contaminated, the quarter IMI status for that day was deemed to be unknown (i.e., missing).Samples that did not fit into either the positive or negative IMI definition were excluded from further analysis. Interpretation of duplicate quarter-milk samples in series results in decreased sensitivity but higher specificity for identifying non-*aureus* staphylococci intramammary infections as compared to a single sample (Dohoo et al., 2011). This approach was chosen to maximize the specificity of culture to identify quarters as positive for a non-*aureus* staphylococci IMI (i.e., minimize false positives).

*Speciation of bacterial isolates*

Isolates cultured from quarters meeting the IMI definition were selected and grown in pure culture on blood agar. Standard benchtop tests were done to presumptively identify bacteria following NMC procedure guidelines, including differential growth on selective media, colony morphology, hemolytic pattern, catalase reaction, Gram stain, and coagulase testing (NMC, 2017). Isolates were preserved in tryptic soy broth with a final concentration of 15% glycerol in cryovials and stored at -80°C. Frozen isolates were sent overnight on ice to the University of Missouri for speciation using MALDI-TOF mass spectrometry (Microflex, Bruker Daltonics) with Flex Control software (Bruker Daltonics). The protocol for identifying bacterial isolates with MALDI-TOF mass spectrometry has been described previously (Adkins et al., 2022). Briefly, generated spectra were assigned a score based on similarity with spectra in the manufacturer’s database (BDAL- 1829023 MBT Compass Library, revision F(8468), version 9), as well as the University of Missouri laboratory custom database (Adkins et al., 2018). The confidence levels used for species identification were applied as previously described (Cameron et al., 2017), in which ≥ 1.7 was used for staphylococcal species-level identification and < 1.7 was classified as inconclusive. Suspect staphylococci isolates unable to be identified to the species level and those identified as *Staphylococcus agnetis* or *Staphylococcus hyicus* by MALDI-TOF were speciated using *tuf*gene sequences with a cut-off of 98% identity as previously described (Hwang et al., 2011).

*Selection and description of data set*

Quarter-day observations were included in this study when the bacteriological status of a quarter on a given day could be determined as previously described. A quarter-day observation was included if: 1) a subclinical IMI due to any of the most frequently observed *Staph.* species (≥ 5 observed IMI) in pure culture, *or* was a culture negative quarter; 2) was collected from a cow ≤ 305 days in milk at time of observation; and 3) had an associated quarter-level SCC measurement. (Figure 1)*.*

*Statistical analysis*

The quarter-day somatic cell counts, quarter-day IMI status, cow parity and DIM data were organized into a spreadsheet (Microsoft Excel, Redmond, WA) and imported into the R Statistical Programming Environment (R Development Core Team, 2023) for analysis. Raw quarter-day-level SCC was converted to SCS [log2(quarter somatic cell count/1000) + 3] in order to address the non-normal distribution of SCC data.

A linear hierarchical repeated measures mixed model was fitted to the data set in order to compare SCS of quarters infected with a single *Staph.* species to healthy quarters. The “lme” function of the “nlme” package was used to build this model, in which the SCS of a quarter on a given day was the outcome variable, and the quarter-day IMI status (with healthy quarters as the reference value) was the main fixed predictor. Interaction between parity and quarter-day IMI status was evaluated to allow the effect of a given IMI to vary as function of age. Similarly, interaction between DIM (as a third degree polynomial variable) and quarter-day IMI status was evaluated to allow the effect of a given IMI to vary as function of DIM. Interaction terms were removed whenever the F-test for these terms yielded a *P*-value < 0.05. Finally, if the DIM by quarter-day IMI status interaction was not significant, then DIM was still kept as a fixed predictor in the model (again as a third degree polynomial variable), but not as part of an interaction, to allow to adjust our SCS estimates as function of DIM.

The hierarchical structure of the data was addressed by fitting random intercepts for quarter, cow, and herd (observations nested within quarter, quarters nested within cow, and cow within herd). Samples collected at different time points for a given quarter were considered repeated measurements, and a spatial exponential correlation structure was used to account for both the correlation between milk samples collected on the same quarter, and for the variation of this correlation with the varying amount of time between sample collections. The model (without interaction) was:

SCS*ijkl* = β0 + β1 Q-D-IMI status*ijkl* + β2DIM*ijkl* + β3DIM*ijkl*2 + β4DIM*ijkl*3 + v*l* + u*kl* + w*jkl* + e*ijkl*,

where SCS*ijkl* is the predicted SCS for the *i*th sample of the *j*th quarter of the *k*th cow from the *l*th herd; β0 is the intercept; β1, β2, β3, and β4 are the regression coefficients for quarter-day IMI status, and DIM as a third degree polynomial variable (to correct for the nonlinear relationship between DIM and SCS); and *vl*, *ukl*, *wjkl*, and *eijkl*are the herd random effect, cow random effect, quarter repeated effect, and sample error term, respectively (approximate normal distribution assumed). Statistical significance was determined using an F-test for interaction terms and a t-test for fixed effects, with significance declared at *P* ≤ 0.05. Final model fit was assessed by checking the homoscedasticity and normality of residuals (graphing of residuals vs. predicted values and Q-Q plots, respectively).

***Results***

*If we report the contamination rate in this paper then, insert a sentence describing how many quarters were samples from how many cows (total duplicate samples taken before eliminating the contaminated samples)*. Collection of a large number of samples in the field under time pressure and occasionally with minimally trained personnel resulted in a moderately high rate of contamination (13%).

Participating herds milked an average of 69.5 cows (median: 70; range: 44-105) of various breeds. Three visits were completed at 8 farms, 1 herd was sampled twice, and 1 was sampled 4 times before interruption by the COVID-19 pandemic. On average, 33.6 days elapsed between sequential farm visits for each herd (median: 34; range: 27-43).

The initial data set included 3,331 quarter-level observations, with 22 different species of staphylococci and mammaliicocci identified. *Staphylococcus* and *Mammaliicoccus* species causing IMI excluded from further analyses due to having < 5 IMI observations included: *M. fleurettii, M. sciuri, M. vitulinus, S. auricularis, S. capitis, S. cohnii, S. epidermidis, S. gallinarum, S. hominis, S. pseudintermedius, S. saprophyticus,* and *S. succinus*. The final data set consisted of 2,260 observations: 648 quarters with an IMI due to 10 different SaM (each causing at least 5 IMI), and 1,972 healthy quarters. Observations included in the final data set came from 1,272 quarters of 360 cows across all 10 herds included in the field study. The mean (median; range) number of cows included per herd was 36 (36; 34-39), whereas the number of quarters included per cow was 3.5 (2; 1-4). The mean number of observations per quarter included was 2.1 (2; 1-4). Twenty-seven percent of observations were the sole observation contributed to the data set by a given quarter, 41% came from quarters contributing 2 time points, and 31% and 1% came from quarters contributing 3 and 4 observations, respectively. The average time elapsed between sequential observations of a quarter was 37.1 days (median: 34.5; SD: 11.6), with an overall range of 27-96 days.

*Staph. chromogenes* was the most frequent species (59% of IMI quarter observations), followed by *Staph. aureus* (17%)*, Staph. haemolyticus* (6%)*,* and *Staph. simulans* (5%)*.* A large amount of variability was observed in the SCS for healthy quarters and those infected with a number of different *Staph.* species, especially *S. chromogenes,* *S.* *haemolyticus, S. simulans,* and *S. aureus* (observed quarter SCS data presented in Figure 2). The observed SCS for *S. chromogenes* IMI ranged from -2.6 to 8.9 (median: 3.3; equivalent to 2,000 cells/mL to 6.1 million cells/mL), with 29.7% of observations having a SCS ≥ 4.0. The observed SCS for *S. aureus* IMI ranged from 0.6 to 10.5 (median: 5.9; equivalent to 8,000 cells/mL to 18 million cells/mL), with 87.5% of observations of having an SCS ≥ 4.0. *Add haemolyticus and simulans data summary here?*

The final model comparing SCS of quarters infected with SaM to healthy quarters and adjusted for DIM is presented in Table 1. Somatic cell score was significantly higher in quarters infected with *S. agnetis, S. aureus, S. chromogenes, S. devriesei, S. haemolyticus, S, hyicus, S. simulans, S. warneri, and S. xylosus* compared to uninfected quarters (Table 1). The interaction between IMI status and DIM was not significant (*P* = 0.42). The effect of parity on SCS was visualized using the raw data, and appeared to have a positive linear relationship with SCS. When SCS was plotted as function of IMI status by parity using the raw data, most bacterial species (with the exception of *S. hyicus,* n = 6 observations) had a relatively constant effect on SCS regardless of parity. A model with an interaction term between IMI status and parity found that the interaction between IMI status and parity was not significant (*P* = 0.86), but parity and bacterial species separately were both significant predictors of SCS (*P* < 0.001). A model was attempted with a three-way interaction term between DIM (3-degree polynomial term), IMI status, and parity, but would not converge due to complete data separation. An additive model with DIM (3-degree polynomial term), IMI status, and parity found all three variables to be statistically significant (*P* < 0.001) predictors of SCS. When compared to the model with only DIM and IMI status, the model including parity changed the coefficients for each *Staph.* sp. group by ≤ 5%, and standard errors by ≤ 1%. As the effect of parity was the same across all groups of IMI status, and the impact of its inclusion was minimal on the coefficients of the variable of interest, only results from the model including DIM (3-degree polynomial term) and IMI status on quarter SCS are presented in the interest of simplicity.

Least square means estimates of quarter SCS across DIM for the ten different SaM modeled as compared to healthy quarters are presented in Figure 3. Estimates for each species are presented for the observed range of DIM available from included quarter-milk samples. Infection by most SaMspecies led to elevation of quarter-milk SCS notably above the SCS of healthy quarters (Figure 3).

Predicted raw SCC for quarters infected with different *Staph.* species at 91 days in milk are presented in Table 2. The highest cell count was for quarters infected with *S. warneri,* followed by *S. aureus, S. agnetis,* and *S. hyicus* (Table 2)*.* Intramammary infection with the most frequent species, *S. chromogenes,* resulted in a quarter somatic cell count of 80,376 cells/mL for a quarter of a cow at 91 DIM (Table 2).

***Discussion***

The current study describes how quarter-milk SCS varied as a result of IMI with the most frequently isolated SaM from a longitudinal, cross-sectional study of 10 certified organic dairy farms in Vermont, US. The relative distribution of various SaM and their effect on qSCC was similar to previous studies describing conventionally-managed dairies. *S. chromogenes* was the most frequent species, followed by *S. aureus, S. haemolyticus,* and *S. simulans*. A large amount of variability was observed in qSCC for healthy quarters and those infected with a number of species, especially *S. chromogenes, S. haemolyticus, S. simulans,* and *S. aureus*. SCC was significantly higher in quarters infected *with S. agnetis, S. aureus, S. chromogenes, S. devriesei, S. haemolyticus, S. hyicus, S. simulans, S. warneri*, and *S. xylosus* compared to healthy quarters. The highest cell count was for quarters infected with *S. warneri*, followed by *S. aureus, S. agnetis*, and *S. hyicus*.

*S. chromogenes* was the most frequently identified SaM associated with subclinical IMI on 10 organic dairy herds in Vermont. This is consistent with other studies using genotypic methods or MALDI-TOF for speciation of *Staphylococcus* isolates from both conventional (De Visscher et al., 2016; Condas et al., 2017a; Rowe et al., 2019; Wuytack et al., 2020) and organic (Peña-Mosca et al., 2023) herds in various countries. In contrast to other work focused on SaM epidemiology and similar to Peña-Mosca et al. (2023), we included *S. aureus* IMI data in our analysis. This was motivated by two factors: 1) *S. aureus* has previously been identified as a pathogen of particular concern on organic dairy farms in the US (Ruegg, 2009), and 2) *S. aureus* IMI would serve as a relevant reference category for effect of IMI on SCS (in addition to healthy/no growth negative control quarters). In agreement with Peña-Mosca et al. (2023), the second most frequently isolated *Staph.* species among these ten herds was *S. aureus.* Distribution of the next most frequently found *Staph.* species (in order, *S. haemolyticus, S. simulans, S. agnetis,* *S. warneri*, *S.* *devriesei*) in the current study was most similar to previous work on SaM in the US and Canada (Condas et al., 2017a; Rowe et al., 2019). Interestingly, *S. equorum*, *S. cohnii,* *S. hominis,* and *M. sciuri* were all commonly-found SaM species in Belgian studies (De Visscher et al., 2016; Wuytack et al., 2020), but were infrequently found in the current study and not included in the final data set. As the farms in the current study were all certified organic dairies, the ecology of intramammary pathogens (including the diversity of *Staph.* species found) could potentially differ from that of conventional farms. We suggest this is possible because, in addition to extent of antibiotic use, differences in management factors exist between conventional and organic dairies (Stiglbauer et al., 2013), and various management factors appear to affect the diversity of SaM species found (Dufour et al., 2012; Condas et al., 2017a). However, we found that the relative distribution of various *Staph.* species in this population of small to midsize organic farms was similar to previous studies describing conventionally managed dairies.

Similarly, as the *Staph.* species on these organic farms are under different selective pressures than those causing IMI on conventional farms, there was the potential that a given species may differ in its effect on SCC and interaction with the host. For example, it is unknown if dominant *S. chromogenes* strains differ between conventional and organic herds. Although we did not test this specific hypothesis, we found no evidence that a given species may vary in effect on SCC in the current study. Similar to previous work describing the effect of different *Staph.* species on quarter SCC (using isolates from multiple herds and genotypic methods or MALDI-TOF for identification), most of the frequently found species from this population of organic dairy farms increased qSCC above that of healthy quarters. Although differences in study design preclude direct comparison of species-level effect on SCC across publications, similar general trends were observed. Fry et al. (2014) also found *S. chromogenes, S. simulans, S. xylosus, S. haemolyticus, S. warneri,* and *S.* *hyicus* had a higher quarter SCC than healthy quarters, as well as *S. capitis* and *S. epidermidis,* two species which were not isolated in great enough numbers from milk samples in the current study to be included in the analysis. Isolates used in Fry et al. were a subset of a larger population from quarter-milk samples collected by the Canadian Bovine Mastitis and Milk Quality Research Network, described by Condas et al. (2017b). This larger study also found the same six SaM species previously listed increased quarter SCC above that of healthy quarters, as well as other staphylococci species included in the current study (*S. aureus, S. agnetis*). While Condas et al. (2017b) found *S. equorum* to elevate quarter SCC above that of healthy quarters, the current study did not. The low number of *S. equorum* IMI observations in our study may have limited our ability to observe an effect on qSCC. Of the 17 SaM species included in Condas (2017b), *S. equorum* had the second lowest quarter SCC (40,800 cells/mL); the only species with a lower qSCC was *S. hominis*, which did not differ from healthy quarters (33,300 cells/mL). In the Canadian study, *S. succinus, S. saprophyticus, S. epidermidis, S. cohnii, M. sciuri, S. gallinarum, S. capitis,* and *S. arlettae* were also found to increase quarter SCC above that of healthy quarters; with the exception of *S. arlettae,* these species were isolated from IMI in the current study but were not present in high enough numbers to be included in the analysis. Although the scope of species included in Supré et al. (2011) was more limited, they also found that IMI due to *S. aureus, S. chromogenes, S. xylosus,* and *S. simulans* resulted in a higher SCC than noninfected quarters. One species not previously compared to healthy quarters in these aforementioned studies is *S. devriesei,* which we found significantly elevated quarter SCC above that of no growth quarters. Although the effects on quarter SCC for *Staph.* species on these organic dairies is similar to those previously described on conventional farms, the potential exists for future work comparing virulence factors and antibiotic resistance determinants of SaM isolates causing IMI on conventional vs. organic dairy farms.

The predicted SCC for quarters infected with *S. aureus* stayed above 200,000 cells/mL across the entire range of observed DIM (Figure 3), a cut-off which has been associated with decreased milk production (Shook, 1982; Hand et al., 2012). The ability of *S. aureus* to elevate quarter SCC above this threshold has been well-established (Supré et al., 2011; Taponen et al., 2022; Woudstra et al., 2023). Infection with *S. warneri* also resulted in a quarter SCC above 200,000 cells/mL throughout the range of observed DIM; at 91 DIM, the estimated qSCC was 395,190 cells/mL (95% CI: 148,189 - 1,053,891, Table 2), which was based off 15 quarter observations. This extends the findings of Fry et al., where the geometric mean SCC for quarters with *S. warneri* was 233,200 cells/mL (95% CI: 90,400-601,600), which was based off 9 quarter observations. The small number of isolates for this species likely resulted in the large 95% confidence intervals of predicted SCC for *S. warneri* seen in both studies. For two studies including larger number of observations for *S. warneri,* quarter SCC estimates stayed well below the 200,000 cells/mL cut-off (for 31 observations in Condas et al., 2017: 63,270 cells/mL, 95% CI: 42,010-95,280; for 105 observations in Taponen et al., 2022: 52,000 cells/mL, 95% CI: 38,000–71,000). In the current study, the predicted qSCC for *S. chromogenes, S. agnetis, S. hyicus, S. simulans,* and *S. xylosus* only became elevated over 200,000 cells/mL late in lactation (286, 208, 261, 270, and 281 DIM, respectively). This effect of DIM is not unexpected, given that SCC normally increases even in healthy quarters towards the tail-end of lactation (Schepers et al., 1997). While still elevated significantly above that of healthy quarters, those infected with *S. devriesei* and *S. haemolyticus* stayed below this threshold throughout the range of DIM assessed for each species.

A readily-available, reliable bench-top test has not yet been developed for differentiating SaM species. With the exception of larger milk quality labs and research settings, the best current methods of speciation for SaM (MALDI-TOF, PCR) are not widely available, likely due to a high equipment costs and some technological barriers. Currently, most SaM species are only able to be lumped together as “non-*aureus* staphylococci” by milk quality labs without the resources or infrastructure to speciate isolates (e.g., on-farm culture, veterinary practices), even though it is established that some species are more relevant to udder health than others. Future work towards developing more readily available methods of speciation may better inform treatment decisions for producers, allowing them to treat or cull animals with infections due to more problematic SaM and withhold treatment for those of less concern.

Although the increase in quarter SCC was modest for most of the SaM species observed in the current study, the widespread nature of these intramammary pathogens can still result in sizeable increases in the bulk tank SCC due to a large number of quarters infected in a given herd. Schukken et al. (2009) found that the percentage contribution of SaM IMI to the total number of somatic cells in bulk tank milk was 17.9% for herds with a BTSCC less than 200,000 cells/mL, considerably greater than the contribution from infections with “major mastitis pathogens” in those herds. The consistently high quarter-level prevalence of SaM found in previous work (26%, Condas et al., 2017; 26%, De Visscher et al., 2016; 11.4%, Rowe et al., 2019; 33%, Wuytack et al., 2020) means that taken as a whole, IMI with these bacteria can still negatively affect the overall income of a dairy by preventing producers from achieving quality premiums. Schukken et al. point out that particularly in “herds striving for a low BMSCC [< 200,000 cells/mL],” where major mastitis pathogens have already been controlled, IMI due to SaM are the next target to further improve udder health. These findings are even more applicable today, as the average SCC for dairies in the US continues to decline and more dairies are achieving a low BTSCC. In the US, the milk-weighted geometric BTSCC mean decreased from 227,000 cells/mL in 2009 to 171,000 cells/mL in 2019 (USDA-APHIS, 2021). The cohort of herds enrolled in this study fit the description of herds aspiring towards a low BTSCC, with an average BTSCC of 186,717 cells/mL (median = 163,583; range = 135,000-329,000).

In the observed data, SCS for quarters with an IMI due to *S. chromogenes* and *S. aureus* had significant overlap; this was similar to work by Woudstra et. al (2022), who reported quarter-level SCC by SaM on one dairy in Sweden. Additionally, Supré et al. (2011) found that *S. chromogenes*, *S. simulans*, and *S. xylosus* induced an increase quarter SCC comparable with that of *Staphylococcus aureus* for 3 farms in the Netherlands, while controlling for DIM, parity, milk production, and herd. More recent research from the same group found that the SCC from quarters with a persistent IMI due to *S. chromogenes* was comparable to SCC of quarters infected with a major pathogen such as *S. aureus* (Valckenier et al., 2021)*.* However, in the current study, this overlap in effect on SCC was no longer apparent for the least square means estimates of quarters infected with *S. aureus* and *S. chromogenes*, which accounted for the effects of DIM and repeated observations.

Within a given *Staph.* species, there was considerable variability in the observed quarter SCC (Figure 2). This within-species variation was also observed by other studies looking at SCC by *Staph.* species, including Fry et al. (2014) and Supré et al. (2011). Quarters with an IMI due to *S. chromogenes* had an especially wide span of observed quarter SCC in the current study, ranging from 2,000 (the lower limit of detection) to 6,100,000 cells/mL. This variability in the effect of *S. chromogenes* on quarter SCC was also noted in Valckenier et al. (2021), where quarters classified as having a transient IMI due to *S. chromogenes* had a mean SCC of 69,000 cells/mL, while those classified as having a persistent *S. chromogenes* IMI had a SCC of 351,000 cells/mL. Wuytack et al. (2020) found *S. chromogenes* to be the most prevalent SaM species causing IMI in quarters identified both as healthy (≤ 50,000 cells/mL) and infected, but with no observable clinical signs (> 50,000 cells/mL), as well as one of the three most common species in quarters exhibiting clinical signs of mastitis. Similarly, Condas et al. (2017b) found that in SaM-positive quarters, *S. chromogenes* was isolated with similar frequency from quarters classified as low-SCC (< 200,000 cells/mL), high SCC (> 200,000 cells/mL), and those with clinical mastitis. This observed diversity in the effect of *S. chromogenes* may suggest that strain type could play a role in the variable pathogenicity of SaM species, as some previous work suggests (Hyvönen et al., 2009; Åvall-Jääskeläinen et al., 2013; Naushad et al., 2019). More work exploring the possible effect of strain type while accounting for cow-level effects (i.e., immune response, DIM, parity), especially for *S. chromogenes*, is warranted to further understand this variability of observed effect on quarter SCC. As we further understand the ecology and epidemiology of individual SaM species and identify species or strains with host-adapted or contagious behavior, speciation and strain typing for SaM will be important as a part of mastitis control decision making.

A large amount of variability was also seen in the observed qSCC for healthy quarters included in the study, which ranged from 2,000 (lower limit of detection) to 8,400,000 cells/mL. The presence of some relatively high quarter SCC observations in this group likely highlights the limitation of using bacterial culture as a method for identifying the quarter IMI status, as was pointed out by Fry et al. (2014). Researchers in that study point out that the low sensitivity of bacterial culture as a test for IMI may have resulted in the presence of some undiagnosed IMI in the healthy quarters. The definition for an IMI in the current study (duplicate milk samples interpreted in series) results in an even lower sensitivity than used by Fry et al., which may compound this issue. However, for a quarter to be considered culture negative in the current study, both milk samples were required to have either no growth at all or no significant growth on both plates, which is also a fairly strict definition. Despite this limitation, the median (Figure 2) and mean (Table 2) SCC for the negative control quarters was still well below that of most SaM species.

Strain typing was not carried out on all isolates of the same species causing IMI in a given quarter (to check that repeated observations of the same species was indeed a persistent infection), as our objective was to identify the effect on SCC by individual SaM species and not to characterize species-level persistence. As finding the same SaM species in a given quarter on different occasions is likely insufficient evidence for a persistent infection (Dufour et al., 2012), it is possible that different strains of the same *Staph.* species have been clustered together in the analysis as repeated observations of a persistent IMI. This may introduce biases in our analysis if an unaccounted for interaction exists between persistency and effect on SCC at the strain level for some *Staph.* species. This is a current gap in our knowledge and an opportunity for future research (De Buck et al., 2021). The majority of positive IMI quarters with repeated observations in the current study were *S. chromogenes*, which has been demonstrated to be a highly persistent intramammary pathogen (Piessens et al., 2011; Valckenier et al., 2021). In unpublished data from Fry et al. (2014), 90% of quarters where *S. chromogenes* was isolated at multiple time points were confirmed to be persistent infections. The second-most common type of IMI in the current study with repeated observations in a given quarter was *S. aureus,* an intramammary pathogen whose ability to cause persistent infections has been well described (Lam et al., 1996; Woudstra et al., 2023). Based on previous findings, we can only speculate that the majority of repeated observations of *S. chromogenes* or *S. aureus* IMI in the current study in a given quarter were persistent infections with the same strain. Notably, the inclusion of random effects for quarter and cow in the model controlled for these important host-level effects on quarter SCC.

***Conclusions***

The current study describes the species-specific effect of intramammary infection with staphylococci on quarter somatic cell count for a population of organic dairies. The diversity of SaM species observed on these 10 organic dairy herds and the species-level effect on qSCC was similar to previous studies in conventional herds. *S. chromogenes* was the most frequently found species, followed by *S. aureus, S. haemolyticus,* and *S. simulans.* Compared to culture healthy quarters, qSCC was higher in quarters infected with 9 of 10 SaM species identified. The highest cell count was for quarters infected with *S. warneri,* followed by *S. aureus, S. agnetis,* and *S. hyicus.* A large amount of variability was observed in qSCC for quarters infected with *S. chromogenes*, *S.* *haemolyticus, S. simulans,* and *S. aureus.* Although the increase in qSCC was modest for most SaM species observed, the widespread nature of these intramammary pathogens can still result in sizeable increases in bulk tank SCC.

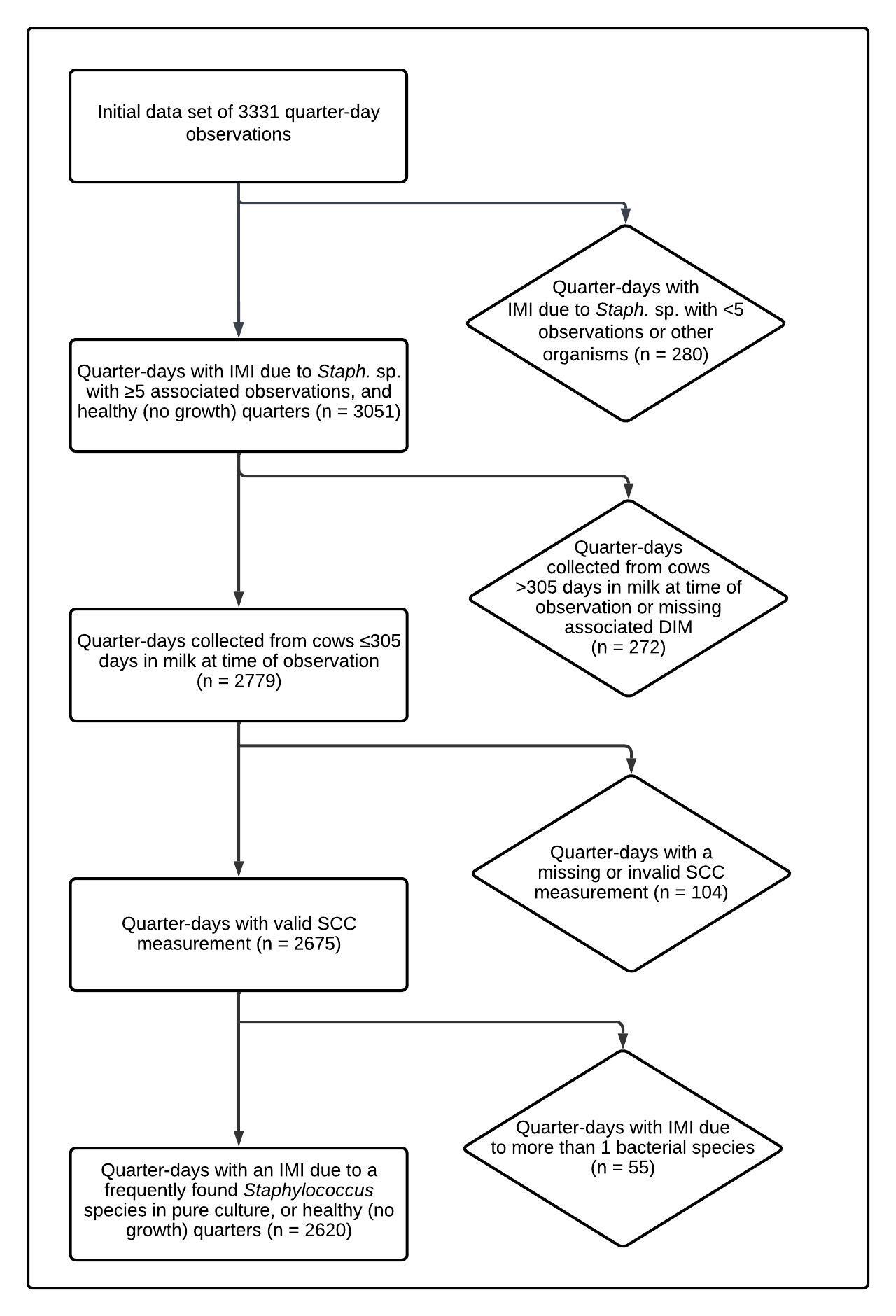


Figure 1.Flow diagram describing selection of final data set of quarter-day observations collected from 382 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US).

|  |  |  |  |
| --- | --- | --- | --- |
| Table 1. Final multivariable model describing the effect of intramammary infection with frequently isolated *Staphylococcus* species on quarter somatic cell score, adjusted for days in milk at time of sampling. Data set is comprised of 2,620 quarter-day observations collected from 1,272 quarters belonging to 360 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US). | | | |
| *Fixed effects* | | | |
| *Staphylococcus* sp. | No. quarter observations | Coefficient estimate (SE) | *P*-value |
| Intercept | - | -0.03 (0.29) | 0.90 |
| *S. warneri\** | 15 | 5.18 (0.60) | < 0.001 |
| *S. aureus\** | 112 | 4.81 (0.22) | < 0.001 |
| *S. agnetis\** | 21 | 3.76 (0.45) | < 0.001 |
| *S. hyicus\** | 6 | 3.23 (0.85) | < 0.001 |
| *S. simulans\** | 35 | 3.11 (0.39) | < 0.001 |
| *S. xylosus\** | 11 | 2.96 (0.62) | < 0.001 |
| *S. chromogenes\** | 384 | 2.88 (0.12) | < 0.001 |
| *S. haemolyticus\** | 40 | 1.77 (0.31) | < 0.001 |
| *S. devriesei\** | 15 | 1.62 (0.54) | 0.003 |
| *S. equorum* | 9 | 0.12 (0.48) | 0.81 |
| Healthy (no growth) | 1972 | *Reference* | *Reference* |
| Days in milk | - | -0.003 (0.01) | 0.54 |
| Days in milk2 | - | < 0.001 (< 0.001) | 0.73 |
| Days in milk3 | - | < 0.001 (< 0.001) | 0.53 |
| *Random effects* | Variance |  | |
| Farm | 0.28 |  | |
| Cow | 1.0 |  | |
| Quarter | 0.47 |  | |
| \* Quarter somatic cell score differs from healthy quarters (*P* ≤ 0.05) | | | |

|  |  |  |
| --- | --- | --- |
| Table 2. Estimated quarter somatic cell count by intramammary infection status at 91 days in milk (13 weeks) for frequently isolated *Staphylococcus* species and healthy (no growth) quarters. Data set used to make model estimations is comprised of 2,620 quarter-day observations collected from 1,272 quarters belonging to 360 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US). | | |
| Quarter-day IMI status | Estimated quarter somatic cell count (cells/mL) | 95% lower and upper confidence level (cells/mL) |
| *S. warneri* | 395,190 | 148,189 - 1,053,891 |
| *S. aureus* | 307,101 | 197,323 - 477,951 |
| *S. agnetis* | 148,437 | 69,021 - 319,232 |
| *S. hyicus* | 102,478 | 26,368 - 398,281 |
| *S. simulans* | 94,617 | 48,346 - 185,175 |
| *S. xylosus* | 84,985 | 30,798 - 234,512 |
| *S. chromogenes* | 80,376 | 56,942 - 113,454 |
| *S. haemolyticus* | 37,333 | 21,217 - 65,688 |
| *S. devriesei* | 33,513 | 13,597 - 82,599 |
| *S. equorum* | 11,855 | 5,292 - 26,556 |
| Healthy (no growth) | 10,927 | 8,056 - 14,822 |

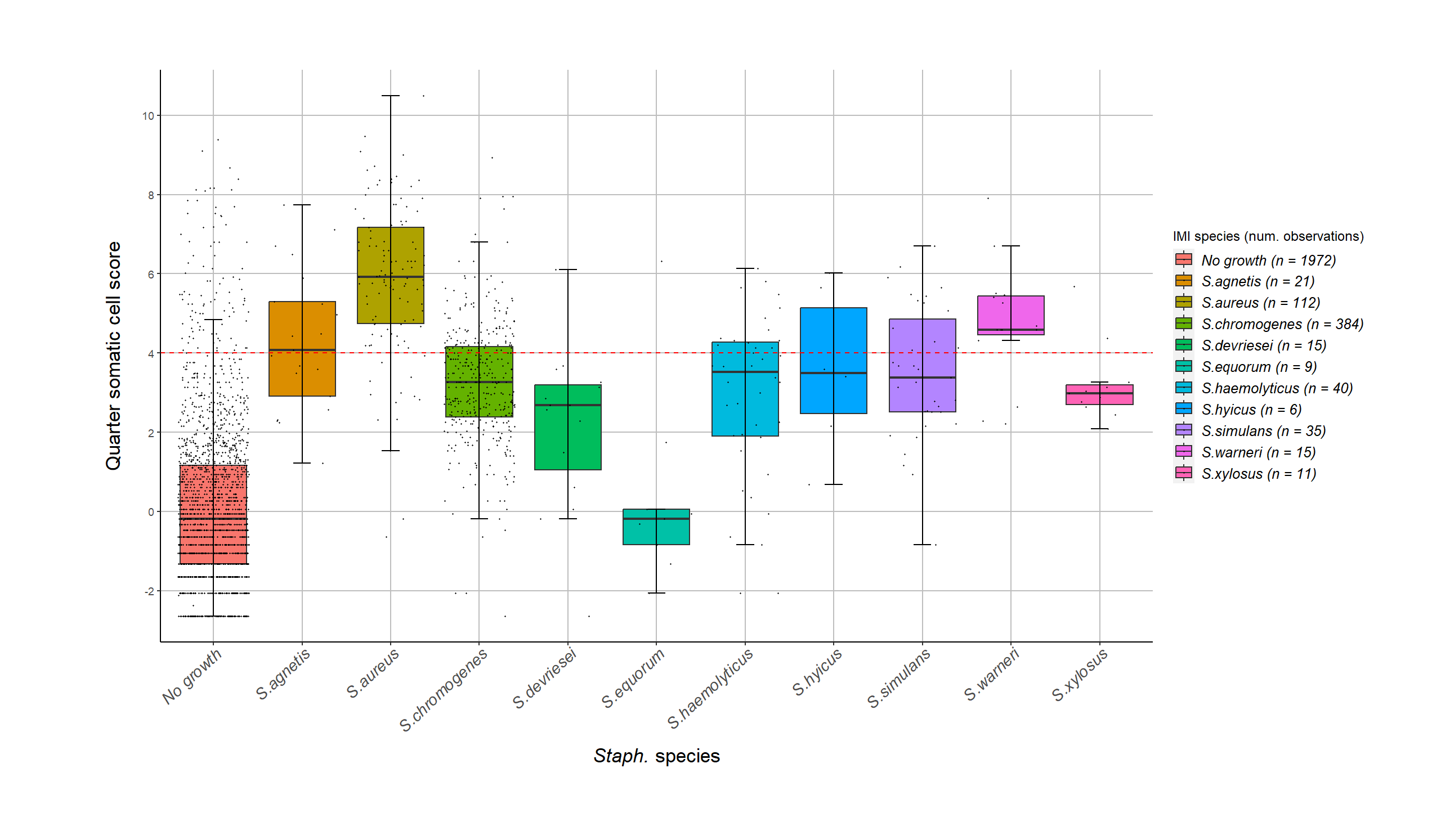


Figure 2. Somatic cell score for 2,260 quarter-day observations with an intramammary infection due to *Staphylococcus* species and healthy (no growth) quarters. Quarter-day observations were collected from 1,272 quarters belonging to 360 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US). The red dotted line indicates a somatic cell score of 4. The observed data are displayed (i.e., quarters that were repeatedly positive for the same species contributed several SCC measurements). Each box contains 50% of the data for a species, the median (line), and is bounded by the 25th and 75th percentiles. The upper whisker represents the largest observation less than or equal to the 75th quartile plus 1.5 times the interquartile range, while the lower whisker represents the smallest observation greater than or equal to the 25th quartile minus 1.5 times the interquartile range.



Figure 3. Quarter somatic cell score least square means estimates as a function of *Staph.* species IMI and days in milk, compared to healthy (no growth) quarters. Data set used to make model estimations is comprised of 2,620 quarter-day observations collected from 1,272 quarters belonging to 360 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US). Model estimates for each species are only presented for the range of days in milk for IMI observations in the data set. Error bars represent the 95% confidence interval.

Adkins, P. R. F., S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton. 2018. Molecular characterization of non-aureus *Staphylococcus* spp. from heifer intramammary infections and body sites. J. Dairy Sci. 101(6):5388-5403.

Adkins, P. R. F., L. M. Placheta, M. R. Borchers, J. M. Bewley, and J. R. Middleton. 2022. Distribution of staphylococcal and mammaliicoccal species from compost-bedded pack or sand-bedded freestall dairy farms. J Dairy Sci 105(7):6261-6270.

Åvall-Jääskeläinen, S., J. Koort, H. Simojoki, and S. Taponen. 2013. Bovine-associated CNS species resist phagocytosis differently. BMC Veterinary Research 9(1):227.

Bombyk, R. A., A. L. Bykowski, C. E. Draper, E. J. Savelkoul, L. R. Sullivan, and T. J. Wyckoff. 2008. Comparison of types and antimicrobial susceptibility of *Staphylococcus* from conventional and organic dairies in west-central Minnesota, USA. J Appl Microbiol 104(6):1726-1731.

Breyne, K., S. De Vliegher, A. De Visscher, S. Piepers, and E. Meyer. 2015. Technical note: a pilot study using a mouse mastitis model to study differences between bovine associated coagulase-negative staphylococci. J Dairy Sci 98(2):1090-1100.

Cameron, M., H. W. Barkema, J. De Buck, S. De Vliegher, M. Chaffer, J. Lewis, and G. P. Keefe. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. J. Dairy Sci. 100(3):2137-2147.

Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, S. Naushad, S. De Vliegher, R. N. Zadoks, J. R. Middleton, S. Dufour, J. P. Kastelic, and H. W. Barkema. 2017a. Prevalence of non-aureus staphylococci species causing intramammary infections in Canadian dairy herds. J Dairy Sci 100(7):5592-5612.

Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, J. P. Roy, G. P. Keefe, T. J. DeVries, J. R. Middleton, S. Dufour, and H. W. Barkema. 2017b. Distribution of non-aureus staphylococci species in udder quarters with low and high somatic cell count, and clinical mastitis. J Dairy Sci 100(7):5613-5627.

De Buck, J., V. Ha, S. Naushad, D. B. Nobrega, C. Luby, J. R. Middleton, S. De Vliegher, and H. W. Barkema. 2021. Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Frontiers in Veterinary Science 8.

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(8):6457-6469.

De Visscher, A., S. Piepers, F. Haesebrouck, K. Supre, 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(1):629-642.

Dohoo, I., S. Andersen, R. Dingwell, K. Hand, D. Kelton, K. Leslie, Y. Schukken, and S. Godden. 2011. Diagnosing intramammary infections: Comparison of multiple versus single quarter milk samples for the identification of intramammary infections in lactating dairy cows. J. Dairy Sci. 94(11):5515-5522.

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(6):3110-3124.

Fergestad, M. E., F. Touzain, S. De Vliegher, A. De Visscher, D. Thiry, C. Ngassam Tchamba, J. G. Mainil, T. L’Abee-Lund, Y. Blanchard, and Y. Wasteson. 2021. Whole Genome Sequencing of Staphylococci Isolated From Bovine Milk Samples. Frontiers in Microbiology 12.

França, A., V. Gaio, N. Lopes, and L. D. R. Melo. 2021. Virulence Factors in Coagulase-Negative Staphylococci. Pathogens 10(2):170.

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(4):2247-2257.

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(8):4876-4885.

Hand, K. J., A. Godkin, and D. F. Kelton. 2012. Milk production and somatic cell counts: A cow-level analysis. J. Dairy Sci. 95(3):1358-1362.

Heikkilä, A. M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. J. Dairy Sci. 101(10):9493-9504.

Hwang, S. M., M. S. Kim, K. U. Park, J. Song, and E. C. Kim. 2011. Tuf gene sequence analysis has greater discriminatory power than 16S rRNA sequence analysis in identification of clinical isolates of coagulase-negative staphylococci. J Clin Microbiol 49(12):4142-4149.

Hyvönen, P., S. Käyhkö, S. Taponen, A. von Wright, and S. Pyörälä. 2009. Effect of bovine lactoferrin on the internalization of coagulase-negative staphylococci into bovine mammary epithelial cells under in-vitro conditions. J Dairy Res 76(2):144-151.

Jenkins, S. N., E. Okello, P. V. Rossitto, T. W. Lehenbauer, J. Champagne, M. C. T. Penedo, A. G. Arruda, S. Godden, P. Rapnicki, P. J. Gorden, L. L. Timms, and S. S. Aly. 2019. Molecular epidemiology of coagulase-negative *Staphylococcus* species isolated at different lactation stages from dairy cattle in the United States. PeerJ 7:e6749.

Lam, T. J., M. C. DeJong, Y. H. Schukken, and A. Brand. 1996. Mathematical modeling to estimate efficacy of postmilking teat disinfection in split-udder trials of dairy cows. J Dairy Sci 79(1):62-70.

National Mastitis Council. 2017. Laboratory Handbook on Bovine Mastitis. Third ed. National Mastitis Council, Inc., New Prague, MI.

Naushad, S., S. A. Naqvi, D. Nobrega, C. Luby, P. Kastelic John, W. Barkema Herman, and J. De Buck. 2019. Comprehensive Virulence Gene Profiling of Bovine Non-aureus Staphylococci Based on Whole-Genome Sequencing Data. mSystems 4(2):10.1128/msystems.00098-00018.

Nyman, A. K., C. Fasth, and K. P. Waller. 2018. Intramammary infections with different non-aureus staphylococci in dairy cows. J. Dairy Sci. 101(2):1403-1418.

Peña-Mosca, F., C. Dean, V. Machado, L. Fernandes, P. Pinedo, E. Doster, B. Heins, K. Sharpe, T. Ray, V. Feijoo, A. Antunes, C. Baumann, T. Wehri, N. Noyes, and L. Caixeta. 2023. Investigation of intramammary infections in primiparous cows during early lactation on organic dairy farms. J Dairy Sci 106(12):9377-9392.

Persson Waller, K., A. Aspán, A. Nyman, Y. Persson, and U. Grönlund Andersson. 2011. CNS species and antimicrobial resistance in clinical and subclinical bovine mastitis. Veterinary Microbiology 152(1-2):112-116.

Piessens, V., E. Van Coillie, B. Verbist, K. Supre, 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(6):2933-2944.

Pol, M. and P. L. Ruegg. 2007. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. J Dairy Sci 90(1):262-273.

R Development Core Team. 2023. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rowe, S. M., S. M. Godden, E. Royster, J. Timmerman, B. A. Crooker, and M. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. J Dairy Sci 102(12):11384-11400.

Ruegg, P. L. 2009. Management of mastitis on organic and conventional dairy farms. J Anim Sci 87(13 Suppl):43-55.

Schepers, A. J., T. J. Lam, Y. H. Schukken, J. B. Wilmink, and W. J. Hanekamp. 1997. Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J Dairy Sci 80(8):1833-1840.

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(1-2):9-14.

Schutz, M. M., L. B. Hansen, G. R. Steuernagel, and A. L. Kuck. 1990. Variation of Milk, Fat, Protein, and Somatic Cells for Dairy Cattle1. J. Dairy Sci. 73(2):484-493.

Shook, G. E. 1982. Approaches to summarizing somatic cell count which improve interpretability. Page 150 in Proc. 21st Annual Mtg. Natl. Mastitis Council, Arlington, VA.

Simojoki, H., T. Orro, S. Taponen, and S. Pyorala. 2009. Host response in bovine mastitis experimentally induced with *Staphylococcus chromogenes*. Veterinary Microbiology 134(1-2):95-99.

Stiglbauer, K. E., K. M. Cicconi-Hogan, R. Richert, Y. H. Schukken, P. L. Ruegg, and M. Gamroth. 2013. Assessment of herd management on organic and conventional dairy farms in the United States. J. Dairy Sci. 96(2):1290-1300.

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(5):2329-2340.

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(7):3301-3307.

Taponen, S., V. Myllys, and S. Pyörälä. 2022. Somatic cell count in bovine quarter milk samples culture positive for various *Staphylococcus* species. Acta Veterinaria Scandinavica 64(1).

Tikofsky, L. L., J. W. Barlow, C. Santisteban, and Y. H. Schukken. 2003. A comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* in organic and conventional dairy herds. Microb Drug Resist 9 Suppl 1:S39-45.

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(5):3071-3078.

USDA-APHIS. 2021. Determining U.S. Milk Quality Using Bulk-Tank Somatic Cell Counts, 2019. Accessed April 2, 2024. <https://www.aphis.usda.gov/sites/default/files/btscc_2019infosheet.pdf>.

Valckenier, D., S. Piepers, A. De Visscher, R. M. Bruckmaier, and S. De Vliegher. 2019. Effect of intramammary infection with non-aureus staphylococci in early lactation in dairy heifers on quarter somatic cell count and quarter milk yield during the first 4 months of lactation. J Dairy Sci 102(7):6442-6453.

Valckenier, D., S. Piepers, A. De Visscher, and S. De Vliegher. 2020. The effect of intramammary infection in early lactation with non-aureus staphylococci in general and *Staphylococcus chromogenes* specifically on quarter milk somatic cell count and quarter milk yield. J Dairy Sci 103(1):768-782.

Valckenier, D., S. Piepers, Y. H. Schukken, A. De Visscher, F. Boyen, F. Haesebrouck, and S. De Vliegher. 2021. Longitudinal study on the effects of intramammary infection with non-aureus staphylococci on udder health and milk production in dairy heifers. J Dairy Sci 104(1):899-914.

Verbeke, J., S. Piepers, K. Supré, and S. De Vliegher. 2014. Pathogen-specific incidence rate of clinical mastitis in Flemish dairy herds, severity, and association with herd hygiene. J. Dairy Sci. 97(11):6926-6934.

Woudstra, S., N. Wente, Y. Zhang, S. Leimbach, M. K. Gussmann, C. Kirkeby, and V. Krömker. 2023. Strain diversity and infection durations of *Staphylococcus* spp. and *Streptococcus* spp. causing intramammary infections in dairy cows. J Dairy Sci 106(6):4214-4231.

Wuytack, A., A. De Visscher, S. Piepers, F. Boyen, F. Haesebrouck, and S. De Vliegher. 2020. Distribution of non-aureus staphylococci from quarter milk, teat apices, and rectal feces of dairy cows, and their virulence potential. J Dairy Sci 103(11):10658-10675.