***Discussion***

Consistent with other studies on 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 using genotypic methods or MALDI-TOF for speciation, *S. chromogenes* was the most frequently identified NASM species associated with subclinical IMI. Although many studies describing frequency of *Staph.* species isolated are understandably focused on NASM, in agreement with Peña-Mosca et al. (2023) the second most-isolated species was *S. aureus.* Distribution of the next most commonly-found species (in order, *S. haemolyticus, S. simulans, S. agnetis,* *S. warneri*/*S.* *devriesei*) was most similar to previous work on NASM in the U.S. and Canada (Condas et al., 2017a; Rowe et al., 2019). Interestingly, *S. equorum*, *S. cohnii,* *S. hominis,* and *M. sciuri* were all commonly-found NASM 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 are all certified organic dairies, it is interesting to note that the ecology of intramammary pathogens on organic farms had the potential to differ from that of conventional farms, as there is no use of antibiotic treatments in a routine manner at dry off or during lactation. However, we found that this was not the case; 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 farms are under different selective pressures than those causing IMI on conventional farms, there was the potential that the same species may differ in their potential virulence and interaction with the host. Again, we found this not to be the case; 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 commonly-found species from this population of organic dairy farms increased qSCC above that of culture negative quarters. 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 negative control 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 quartermilk 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 NAS species increased quarter SCC above that of culture negative quarters, as well as other staphylococci species included in the current study (*S. aureus, S. agnetis*). While Condas et al. (2017) found *S. equorum* to elevate quarter SCC above that of negative quarters, the current study did not. Of the 17 NAS species they included, *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 culture negative quarters (33,300 cells/mL). It may be that overall qSCC for culture negative quarters in the current study was higher than that of Condas et al., which could preclude finding a relatively small difference in SCC between the two groups. 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 negative control 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 negative quarters in these aforementioned studies is *S. devriesei,* which we found significantly elevated quarter SCC above that of negative control 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 NASM 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 in agreement with 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). [Has anyone else looked at SCC effect late vs. earlier in lactation? Effect of these compounded/exacerbated by being late lactation?] While still elevated significantly above that of culture negative quarters, those infected with *S. devriesei* and *S. haemolyticus* stayed below this threshold throughout the range of DIM assessed for each species. Unlike a coagulase test for *S. aureus,* a readily-available, (mostly) reliable bench-top test has not yet been developed for differentiating NASM species. With the exception of larger milk quality labs and research settings, the best current methods of speciation for NASM (MALDI-TOF, PCR) are not widely used due to a high cost and technological barrier. Currently, most NASM species are only able to be lumped together as “non-*aureus* staphylococci” by smaller-scale milk quality labs and producers doing on-farm culture, even though we know some species to be 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 species and withhold treatment for those of less concern.

Although the increase in quarter SCC was modest for most of the NAS species observed in the current study, the widespread nature of these intramammary pathogens can still result in sizeable increases in the bulk tank somatic cell count due to a large number of quarters infected in a given herd. Schukken et al. (2009) found that the percentage contribution of NASM 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 high quarter-level prevalence of NASM (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, intramammary infections 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 in particular, in “herds striving for a low BMSCC [<200,000 cells/mL],” where major mastitis pathogens have already been controlled, IMI due to NASM are the next target in sight to further improve udder health. These findings are even more applicable today, as the average somatic cell count [for dairies in the U.S.] continues to decline and more dairies are achieving a low BTSCC. In the U.S., the milk-weighted geometric BTSCC mean decreased from 227,000 cells/mL in 2009 to 171,000 cells/mL in 2019 (USDA-APHIS, 2021).

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 *Staph.* species 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 (Valckenier et al., 2021) 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.* 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 group, there was considerable variability in the observed quarter SCC. 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 NAS species causing IMI in quarters identified both as healthy (≤50,000 cells/mL) and subclinically infected (>50,000 cells/mL), as well as one of the three most commonly isolated demonstrating clinical signs of mastitis. Similarly, Condas et al. (2017b) found that in NAS-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 NAS 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.

A large amount of variability was also seen in the observed qSCC for culture negative 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 IMI, 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 negative control 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, in order 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 NAS species.

Strain typing was not carried out on all NAS 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 and not reinfection of the same quarter with a different strain of the same species. As finding the same species in a given quarter on different occasions is likely insufficient evidence for a persistent infection (Dufour et al., 2012), this may mean that different strains of the same species of NAS have been clustered together in the analysis as repeated observations of a persistent IMI. However, although strain may vary in these repeated observations, the inclusion of random effects for quarter and cow in the model still control for these important host-level effects on quarter SCC. The majority of positive IMI quarters with repeated observations were *S. chromogenes*. Although strain-typing was not performed on all isolates for the current study, *S. chromogenes* 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. In the current study, it is also likely that the majority of repeated observations of *S. chromogenes* IMI in the same quarter were persistent infections. Preliminary work for a project using the same population of bacterial isolates found that all but one of 75 quarters where *S. chromogenes* was repeatedly isolated (from 2-3 timepoints) had an IMI caused by the same strain type (unpublished data)*.* Sixty-four of these 75 quarters where strain-typing was used to confirm persistent *S. chromogenes* infections are included in the current data set. The second-most common type of IMI 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). Although some repeated observations of quarters with *S. chromogenes* or *S. aureus* in the current study may represent new infections with a different strain type, it is likely that the majority are truly persistent.

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