***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 with the most frequently isolated species. Compared to negative quarters, SCC was higher in quarters infected with 9 of 10 *Staph.* species. Although increase in SCC was modest for most species observed, their widespread nature can still result in sizeable increases in bulk tank SCC.

***Running head:***

*NASM affecting udder health on organic dairies*

***Title***

Non-aureus staphylococci and mammaliicocci: which species are important for udder health on organic dairy farms?

Caitlin E. Jeffrey,1 Pamela Adkins2, Simone Dufour2, John W. Barlow1

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

2 Department of Plant and Soil Science, University of Vermont, Burlington, VT 05405

3 Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108.

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 non-aureus staphylococci and mammaliicocci (NASM) species causing intramammary infections (IMI) 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 identified which NASM species are most relevant to udder health 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 commonly-isolated *Staphylococcus* species. Aerobic culture of quartermilk samples to identify IMI was conducted in parallel with determination of qSCC. A linear hierarchical repeated measures mixed model was used to compare somatic cell scores associated with quarters identified to have IMI with a given *Staph.* species to culture negative quarters, and included days in milk at time of sampling to adjust estimates of the *Staph.* species and qSCC association. The final data set consisted of 648 quarters with an IMI due to 10 different *Staph.* sp. and 1,972 negative quarters. *S. chromogenes* was the most commonly-found species, followed by *aureus, haemolyticus,* and *simulans.* A large amount of variability was observed in the somatic cell score for negative quarters and those infected with a number of *Staph.* species, especially *S. chromogenes* and *aureus.* Somatic cell score was significantly higher in quarters infected with *S. agnetis, aureus, chromogenes, devriesei, haemolyticus, hyicus, simulans, warneri, and xylosus* compared to uninfected quarters. The highest cell count was for quarters infected with *S. warneri,* followed by *aureus, agnetis,* and *hyicus.* The relative distribution of various *Staph.* species 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 NASM species observed, the widespread nature of these intramammary pathogens can still result in sizeable increases in bulk tank SCC. Future work towards developing more readily available methods of speciation may better inform treatment decisions, allowing producers to treat or cull animals with infections due to more problematic species and withhold treatment for those of less concern.

***Keywords:***

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

***Introduction***

The group of bacteria known as non-*aureus* staphylococci and mammaliicocci (NASM) are the predominant pathogens causing intramammary infections in dairy animals globally. Cow-level prevalence in one U.S. study was 71% (Jenkins et al., 2019), and quarter-level prevalences of 11, 26, 21, and 33% have been reported in Canada, the U.S., and Belgium (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), NASM 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, NASM intramammary infections (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 the ability of NASM to increase somatic cell count (SCC) is well-established (Supré et al., 2011; Tomazi et al., 2015; Condas et al., 2017b; Valckenier et al., 2019), as well as their ability to persist for long periods of time in the udder (Piessens et al., 2011; Nyman et al., 2018; Valckenier et al., 2021). For many dairy farms that have implemented modern mastitis control practices minimizing the effects of bacteria considered to be “major” pathogens, the leading contributor to bulk tank milk SCC on farms with good milk quality is mammary gland infections due to NASM (Schukken et al., 2009).

NASM 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 NASM 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). NASM 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, NASM 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).

Of the previous work exploring the species-specific effect of different NASM on udder health, a limited number of studies have described the effect of different 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 (DIM) 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 determination of SCC (Schutz et al., 1990; Schepers et al., 1997). Additionally, 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 overall in many 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). Interestingly, antimicrobial susceptibility of common mastitis pathogens can differ between conventional and organic dairy farms in the U.S. (Tikofsky et al., 2003; Pol and Ruegg, 2007; Bombyk et al., 2008). These differences between conventional and organic farms are significant, as: 1) variation in NASM species distribution and diversity is associated with a variety of different management practices (Dufour et al., 2012; Condas et al., 2017a), and 2) in the absence of antibiotic usage, disparate selective pressures on organic dairies could potentially result in population differences -

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

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.