***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 no 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, only a limited number of studies have described the effect of a diverse number 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 NASM species are most relevant to udder health for a population of exclusively 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 of mastitis-causing bacteria.

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.

*Introduction to the problem (questions, objectives, reasons for research, and related literature)*

*The introduction should concisely describe the rationale for conducting the study, background, objectives, and hypotheses to be tested*

***Objective:***

*In this study, multiple SCC measurements were conducted on quarters from cows from 10 herds in VT, US. In parallel, microbiological analyses were conducted to identify the main non-aureus staphylococci IMI present. The objective of these analyses was to estimate how SCC varied as function of bacterial species involved.*

***Hypothesis?***

*Some Staph. spp. will elevate quarter SCC above that of culture negative quarters?*

***Introduction notes:***

* **(1) Importance of NASM, generally, on udder health?**
  + **Why do we care about them?**
    - How widespread they are
    - Primarily associated with subclinical (vs. clinical) mastitis
      * + Persson-Waller 2011, Heikkila 2018
      * … however, are also capable of causing clinical mastitis
        + Taponen et al., 2007; Simojoki et al., 2009; Verbeke et al., 2014, Wuytack 2020 JDS, Condas 2017
    - As a group, can be persistent
      * + Taponen et al., 2007, Nyman 2018, Valckenier 2021, Piessens 2011
      * However, shown that high percentage can have spontaneous cure rate
        + 39.5% Taponen at al 2007, Valckenier 2020
    - Dufour 2012: “*CNS IMI most often remain subclinical and generally lead to only mild to moderate SCC elevations compared with IMI caused by major mastitis pathogens (Djabri et al., 2002; Sampimon et al., 2010; Supré et al., 2011).”*
    - Condas 2017: “*Researchers have previously concluded that NAS as a group are associated with higher SCC when compared with culture-negative quarters…”*
    - Long been considered a “minor pathogen”
    - NAS as a group increase somatic cell count (SCC)
      * Supre 2011, Tomazi 2015, Condas 2017, Valckenier 2019
    - although generally, subclinical NAS intramammary infections don’t have much effect on milk yield
      * Tomazi 2015, Valckenier 2020
    - Many farms have implemented modern mastitis control practices minimizing the effects of bacteria considered to be “major” pathogens
      * Well-described, effective control practices based on thorough understanding of “major” mastitis pathogens
      * the quintessential examples of this are the organisms Staphylococcus aureus, Streptococcus agalactiae
    - The leading contributor to bulk tank milk somatic cell count on farms with good milk quality is mammary gland infections due to non-aureus staphylococci
      * Schukken 2009
* ***(2) Extremely heterogenous group of bacteria***
  + De Visscher 2017, 25 different species causing IMI; Condas 2017 23 species
  + From Condas 2017: *Good evidence indicates that the various species composing this group do not behave homogeneously and, therefore, can contribute to diverse epidemiological findings (Vanderhaeghen et al., 2014)… Both the distribution and effects on udder health associated with NAS vary.*
  + Differences in virulence, antimicrobial resistance, and interaction with host immune system exist within NAS species (Avall-Jaaskelainen 2013; Breyne 2015; Hyvonen 2009; Mork 2012; Piccart 2016).
  + ***NAS species differ from each other in how they behave as an intramammary pathogen***
    - Probable source (environment, intramammary, skin)
      * “ecology and epidemiology”
      * Within this highly heterogenous group, some species are considered primarily host-adapted (colonizing the skin or udder), while others are primarily found in the cow’s environment (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., 2017), and others with environmental contamination and poor teat hygiene at milking time (De Visscher et al., 2016; De Visscher et al., 2017).
    - Ability to cause persistent infections
      * Nyman 2018, Valckenier 2021
  + ***NAS species differ in their virulence potential (ability for host invasion and tissue damage)***
    - Antimicrobial resistance pattern
      * Frey 2013, Fergestad 2021
    - Virulence
      * Naushad 2019, Franca 2021
    - Interaction with host’s immune system
      * Bryene 2015, Avall-Jääskeläinen, 2013
  + **AAANNNNDDD Degree to which they cause an inflammatory reaction in the udder (measured by quarter SCC)**
    - Condas 2017, Fry 2014, De vischer 2016, Supre 2011, Nyman 2018 (composite SCC), Wuytack 2020 (Nov JDS)
* **(3) So, important to elucidate their effect on qSCC**
  + identification of species as more relevant to udder health (based on qSCC effect)
  + How well *do* we understand the effect of NASM on udder health? Is previous work on this, but limited number
    - Quarter-level (not composite cow level) SCC
    - including multiple herds
    - controlling for DIM
    - ID by MALDI or genotypic method
      * From Fry 2014: “*Unfortunately, the number of studies that have identified CNS isolates to the species level is somewhat limited. Also, comparison of the results between studies is difficult because, until recently, many studies used various phenotypic speciation techniques. Phenotypic speciation methods are poorly adapted for identifying CNS isolates of bovine origin (Zadoks and Watts, 2009). Genotypic speciation methodologies have recently been validated for samples of bovine origin. These methods are increasingly being used in research to differentiate CNS isolates because of higher reproducibility and typeability (Mellmann et al., 2006).”*
    - Organic herds
      * Lastly, herds are organic, providing a unique opportunity to study NAS infection dynamics in a farm setting where the selective pressure of antibiotic use has been removed.
  + New species?
    - Elevation of SCC by many species described, but not all species known to cause IMI… and there are lots!

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