Consumer demand for organic dairy products around the world continues to increase, with the global market for organic dairy reaching $23.9 billion in 2022, and is projected to grow 6.5% between 2023 and 2028 (BusinessWire, 2024). In May 2024, the U.S. sold over 63 million kg of organic whole milk, a 20.2% increase from the previous year (USDA-AMS, 2024). Although ranked 19th for overall production, dairy farming is an incredibly important part of Vermont’s agricultural production; dairy comprised 65% of the state’s total farm sales, the highest in the US for 2023 (Progressive Dairy, 2024). In 2021, at the time of the last USDA Certified Organic Survey, Vermont (US) had 147 organic dairy farms, which made over 85 million kg of fluid milk, worth over 59 million dollars (USDA, 2022).

Differences in both management practices and herd characteristics have been identified between organic farms and conventional farms in the US. Specifically, a study of farms in 3 US states found that organic farms were smaller, produced less milk, were more likely to house cows in tiestalls (vs. freestalls), and exhibited differences in how cows were fed and watered (Zwald et al., 2004). Another study including farms from 3 US states compared farms which were matched for size, and found cows on organic farms were older, fed less grain, and produced less milk (Stiglbauer et al., 2013).

Perhaps the most significant difference in management practices between conventional and organic dairies in the US is that antibiotics are not allowed for use in organically produced milk (USDA, 2024). Antibiotics are a significant component of mastitis control and treatment on conventional farms, limiting options for organic dairy producers have in controlling mastitis (Ruegg, 2009; NMC, 2019). Without the ability to use antibiotic treatments, the potential exists that udder health on organic farms could theoretically be worse than that of conventional farms. However, the differences between the two systems are not clear-cut. At the bulk tank level, organic farms were more likely to be positive for *Staphylococcus aureus,* but less likely to have an increased colony count (Stiglbauer et al., 2013), whereas conflicting findings have been reported for bulk tank SCC (Cicconi-Hogan et al., 2014; Levison et al., 2016). At the cow level, some studies have found that SCC was higher on organic farms (Zwald et al., 2004), while others have found no difference (Hardeng and Edge, 2001; Mullen et al., 2013). A lower level of clinical mastitis has been reported for organic dairies in comparison to conventional (Hamilton et al., 2006; Richert et al., 2013; Levison et al., 2016), although this difference disappeared in Valle et al. (2007) when controlling for the lower milk yield of organic cows. While some work has found no difference in the prevalence of particular pathogens causing IMI between the two systems (*Staph. aureus*, coagulase-negative *Staph.* spp., *Streptococcus* spp., or *Corynebacterium* spp., Mullen et al., 2013), a large US study by Pol and Ruegg (2007) found that the prevalence of most mastitis pathogens, except *Staph. aureus*, differed between organic farms and conventional farms (coagulase-negative *Staph.* spp., *Strep. agalactiae*, *Strep*. spp., coliforms, and “other pathogens”). Taken as a whole, this body of work suggests that differences in mastitis epidemiology may exist between conventional and organic dairy farms.

Organic dairy producers with small- to midsize farms in the Northeastern US have expressed interest in bedded pack systems (BP) as an option to house cows during the non-grazing season, as these facilities integrate well with pasture-based farm systems (Thurgood, 2009; Andrews et al., 2021). Additionally, state and federal agencies in the U.S. are providing financial incentives to build these structures as part of manure management practices which improve water quality and contribute to soil conservation (USDA-NRCS). Currently, most small-midsize organic dairies in Vermont use a tiestall (TS) to house their animals while not on pasture (Andrews et al., 2021). As interest in BP grows among organic farmers, it is important to better understand milk quality, udder health and hygiene on farms using this facility type. Given the importance of organic dairies in Vermont and the continued increase in demand for organic dairy products worldwide, a longitudinal, cross-sectional observational study was undertaken to describe the diversity of species causing IMI on organic dairy farms in the state. The specific objectives of the project were to characterize the prevalence of IMI caused by different microorganisms in lactating dairy cattle on 10 small-midsize organic farms in Vermont, both for farms using the most common type of housing for organic farms in the state (TS) and BP.

Enrolled farms were a non-probability subsample of certified organic dairies which had participated in previous studies and milked 35-120 cows. The study was carried out Winter 2019-2020, with 5 herds enrolled using a TS bedded with shavings/sawdust to house lactating dairy cows, and 5 herds using a BP. The inclusive term “bedded pack” encompasses both aerobically composting bedded packs and deep bedded packs, and was defined as an enclosed loose housing facility deeply bedded with organic material (Jeffrey et al., 2024). Approximately 35 cows in early- to mid-lactation were enrolled from each herd. For 8 herds with DHIA data, cows were stratified by SCC, parity, and DIM, then randomly selected across these variables. All cows were sampled in 1 herd with ~35 lactating cows, and for the remaining herd the producer generated a list of 35 cows in early lactation to be sampled. Cows unable to be sampled at a follow-up visit 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).

Standard aerobic bacteriological culture of quarter-milk was performed in duplicate to identify bacterial species present in the sample according to NMC guidelines (NMC, 2017). Aerobic culture results of both samples were then used together to determine the overall bacteriological status of each quarter-milk sample into the following categories: 1) “no significant growth,” when there was 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; 4) “contaminated,” when ≥ 1 of the 2 samples had more than 2 morphologically distinct isolates growing on a plate; 5) and “indeterminate,” when the set of quarter-milk samples did not meet the criteria for any of the previous categories (e.g., missing duplicate). Quarter-day observations were included in this study when the bacteriological status of a quarter on a given day could be determined.

Isolates from both pure and mixed culture quarter-milk samples were then identified to species or genus using MALDI-TOF mass spectrometry (Microflex, Bruker Daltonics). The protocol for identifying bacterial isolates with MALDI-TOF mass spectrometry has been described previously in Haw et al. (2024). For isolates unable to be identified with MALDI-TOF, other identification methods were used [colony morphology, catalase reaction, Gram stain, PCR-based amplicon sequencing for16S rRNA gene (Weisburg et al., 1991) or *rpob* gene (Drancourt et al., 2004)]. Using the bacteriological status and speciation information, a quarter-day IMI status was assigned to each quarter observation: 1) “healthy,” when there was no significant growth; 2) “single pathogen infection,” when ≥ 100 CFU/mL of a particular pathogen was identified in pure culture on both plates (interpretation in series; Dohoo et al., 2011); 3) “mixed infection,” when ≥ 100 CFU/mL of 2 different pathogens were identified in mixed culture on both plates; and 4) “unknown” if the sample status had been identified as contaminated or indeterminate as previously described. A quarter-day observation was included in the final data set if the IMI status was classified as healthy, single pathogen infection, or mixed infection.

Quarter-day IMI status, cow information, visit, and herd 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. The quarter-level prevalence for each farm visit was calculated by dividing the number of quarters infected with a particular pathogen (or grouping of similar pathogens) by the total number of sampled quarters at risk where IMI status could be determined for that farm visit. Median and range of quarter-level prevalence for each herd was then calculated using all consecutive visits to a particular farm. Median and range of quarter-level prevalence for tiestalls and bedded packs were calculated over all 15 visits to each facility type, respectively. Overall median and range of quarter-level prevalence were calculated using all 30 visits to the 10 farms.

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