***Abstract***

A longitudinal study was undertaken to characterize the prevalence of intramammary infections (IMI) caused by different microorganisms on 10 small-midsize organic farms in Vermont (US), both for farms using tiestalls and farms using bedded packs. Most IMI were caused by non-*aureus* staphylococci. At the species level, *Staph. chromogenes* was the leading cause of IMI, followed by *Strep. uberis* and *Staph. aureus*. The observed diversity of species was similar to the limited research previously describing pathogen-specific prevalence of IMI on organic farms. Quarter-level prevalence of IMI by pathogen was similar between bedded pack and tiestall farms in the study.

***Keywords:*** Mastitis, organic dairy cattle, intramammary infection, bedded pack, tiestall

***Introduction, Methods, and Results***

In May 2024, the US sold over 63 million kg of organic whole milk, a 20.2% increase from 2023 (USDA-AMS, 2024). Although ranked 19th in overall milk production, dairy farming is an incredibly important part of Vermont’s agricultural sector; dairy comprised 65% of the state’s total farm sales in 2023, the highest in the US (Progressive Dairy, 2024). In 2021 (most current USDA Certified Organic Survey), Vermont had 147 organic dairy farms, which made over 85 million kg of fluid milk, worth over $59 million (USDA, 2022).

Differences in both management practices and herd characteristics exist between organic and conventional dairies. Organic farms were found to be smaller, produce less milk, more likely to house cows in tiestalls (TS; vs. freestalls, FS), and exhibited differences in how cows were fed and watered (Zwald et al., 2004). When farms were matched for size, cows on organic farms were older, fed less grain, and produced less milk (Stiglbauer et al., 2013). Perhaps the most significant difference between conventional and organic dairies in the US is that antibiotics are not allowed for use on organic farms (USDA, 2024). Antibiotics are a significant component of mastitis control and treatment on conventional farms, leaving limited available options for organic dairy producers to effectively control mastitis (Ruegg, 2009; NMC, 2019). Although this could potentially result in worse overall udder health on organic farms vs. conventional farms, the differences between the two systems are not clear-cut. At the bulk tank milk 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 somatic cell count (SCC) (Cicconi-Hogan et al., 2014; Levison et al., 2016). At the cow level, some work found SCC was higher on organic farms (Zwald et al., 2004), while others found no difference (Hardeng and Edge, 2001; Mullen et al., 2013). A lower level of clinical mastitis has been reported for organic dairies (Hamilton et al., 2006; Richert et al., 2013; Levison et al., 2016), although this difference disappeared in Valle et al. (2007) when controlling for lower milk production by organic cows. While some research found no difference (Mullen et al., 2013), Pol and Ruegg (2007) found that the prevalence of most mastitis pathogens (except *Staph. aureus*) differed between organic and conventional farms in the US. Overall, research suggests that differences in mastitis epidemiology may exist between conventional and organic dairy farms.

Organic dairy producers with small-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 (Andrews et al., 2021). Additionally, state and federal agencies in the US 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 organic dairies in Vermont use a TS to house their animals while not on pasture (Andrews et al., 2021). As interest in BP grow among organic farmers, it is important to understand any udder health implications for farms using this facility type. Given the continued increase in demand for organic dairy products and the importance of organic dairies to Vermont, a longitudinal 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 for 10 small-midsize organic farms in Vermont, both for farms using TS and farms using 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. Cows unable to be sampled at a follow-up visit were replaced with another lactating cow in the herd (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 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 for 16S rRNA or *rpob* gene). Using the bacteriological status and species identification, 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 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.

Median herd size was 70 lactating cows (range: 44-105) of various breeds, with a median rolling herd average of 13,250 lbs. (range: 10,675-21,204 lbs.). 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. Mean days elapsed between farm visits was 33.6 (median: 34; range: 27-43). Of the 5 enrolled BP farms, 2 were composting BP, cultivating the pack twice a day to encourage aerobic decomposition of sawdust or shavings (The Dairyland Initiative, 2024; Bewley et al., 2017). Two BP used a “traditional” or “deep bedded pack” system, where large volumes of dry straw or hay was added to bedding that accumulated over the 6-8 months cows were housed indoors (The Dairyland Initiative, 2024; Bewley et al., 2017). The remaining BP bedded with straw and woodchips and cultivated every 48 hrs., adding chopped hay and woodchips each time.

In total, 4,212 quarter-observations were collected from 1,536 quarters belonging to 384 cows were enrolled for at least 1 visit. Of these, 880 quarter-observations were excluded from further analyses: 34 did not meet definition of either having an IMI or being healthy; 88 were from non-functional mammary glands; 224 were excluded due to a sampling error; and 534 were excluded because ≥ 1 of the 2 duplicate quartermilk samples was contaminated. The final data set consisted of 3,332 quarter-observations (from 1,456 quarters of 382 cows) where the IMI status of the quarter could be determined. There were 2,290 quarter-observations from healthy quarters. The mean (median; range) number of cows included per herd was 38.2 (38; 35-41), quarters per cow was 3.8 (4; 1-4) and observations per quarter was 2.3 (2; 1-4).

There were 1,042 quarter-observations from quarters with an IMI at time of sampling: 953 with an IMI due to a single pathogen (28.6% of all quarter-observations), and 89 with a mixed infection (2.7% of all quarter-observations). The quarter-level prevalence of pathogens (or grouping of similar pathogens) causing intramammary infections by farm is presented in Table 1. Overall, the majority of IMI were caused by all NASM species combined (median prevalence of 20%). At the species level, *Staph. chromogenes* was the leading cause of IMI (14.6%), followed by *Strep. uberis* (3.4%), *Staph. aureus* (3.2%), and *Staph. haemolyticus* (1.3%).

***Discussion***

*Staphylococcus* were the dominant organisms causing IMI in this population of farms, with the largest proportion of IMI caused by non-aureus staphylococci and mammaliicocci (NASM). The median NASM prevalence in the current study is similar to previous studies reporting a quarter-level prevalence of 26% in Canada (Condas et al., 2017) and 21% in Belgium (Valckenier et al., 2020), although higher than 1 US study (11%, Rowe et al., 2019) and lower than another Belgian study (33%; Wuytack et al., 2020). *Staph. chromogenes* was the most frequently identified species, consistent with other studies from both conventional (De Visscher et al., 2016; Condas et al., 2017; Rowe et al., 2019; Wuytack et al., 2020) and organic (Peña-Mosca et al., 2023) herds in various countries. In agreement with Peña-Mosca et al. (2023), the second most frequently isolated *Staph.* species was *Staph. aureus.* However, the quarter-level prevalence observed in the current study was much lower than the 13.6% reported for the second post-partum sampling of the 5 organic farms in Peña-Mosca et al. (2023). A similar distribution pattern of NASM was observed in both the current study and Peña-Mosca et al. (2023), where a diverse number of species were identified but the prevalence of non-*chromogenes* IMI was low. *Staph. haemolyticus* was found at almost twice the quarter-level prevalence in the current study when compared to Peña-Mosca et al. (2023) (1.3% vs. 0.7%, respectively). While Peña-Mosca et al. (2023) found *Strep. dysgalactiae* to be the dominant streptococcal species vs. *Strep. uberis* (quarter-level prevalence of 4.2% and 0.5% respectively for their second post-partum samples), the relative distribution of these 2 species was reversed in the current study (0.4% for *Strep. dysgalactiae* and 3.4% for *Strep. uberis*).

Farms from the current study exhibited a large amount of variation in quarter-level prevalence of *Corynebacterium* spp. The median prevalence in the current study (0.9%) is similar to a large US study (1.16%; Rowe et al., 2019) and lower than that reported by a large Canadian study of fresh cows (3.2% in first-calf heifers, 4.7% multiparous cows; Naqvi et al., 2018). Three farms in the current study had no *Corynebacterium* spp. isolated from subclinical IMI over all farm visits, 5 had a prevalence ranging from 0.4-4%, 1 TS had a prevalence of 7.5%, and 1 BP had a prevalence of 11.5%. Similar findings have been reported by other studies describing subclinical IMI by pathogen on organic dairies. In a comparison of 7 organic and 7 conventional herds, Mullen et al. (2013) report that percentages of quarters infected with *Corynebacterium* spp. showed high variability for the organic farms, ranging from 0 to 63.5%. Peña-Mosca et al. (2023) also found a relatively high proportion of quarters infected with *Corynebacterium* spp. (2.8-5.4% for various sampling periods post-partum). Research exploring risk factors associated with *Corynebacterium* spp. may identify whether organic farms exhibit a wider range of prevalence for these pathogens, or if this observed herd-level variability is seen in both conventional and organic farms.

Research describing the pathogen-specific prevalence of subclinical mastitis is limited for farms using BP, but Fávero et al. (2015) observed that *Corynebacterium* spp. were the most common cause of subclinical IMI in a study of 3 BP farms in Brazil, followed by coagulase-negative *Staph.* Similar to the current study, Freu et al. (2023) report *Staph. chromogenes* was the dominant cause of subclinical IMI for 7 herds using BP in Brazil. Quarter-level prevalence of *Staph. chromogenes* in that study was 24.9%, followed distantly by *Strep. agalactiae* (5.4%)and *Staph. aureus* (4.1%). For the BP in the current study, the second-most common pathogen identified was *Strep. uberis*, followed by *Staph. haemolyticus.* No *Strep. agalactiae* was found in the current study. A number of NASM species were identified which were unique to TS in the current study, including *Mammaliicoccus sciuri, Staph. auricularis, Staph. capitis, Staph. cohnii, M. fleurettii, Staph. hominis, Staph. pseudintermedius, Staph. saprophyticus,* and *M. vitilinus.* In contrast, *Staph. epidermidis, Staph. gallinarum,* and *Staph. succinus* were only isolated from IMI on BP. Work comparing NASM diversity between BP and TS is limited, but a study comparing bulk tank milk between sand-bedded FS and CBP also found that some species were unique to facility type. Adkins et al. (2022) observed a greater diversity of NASM species in bulk tank milk for FS, including *Staph. capitis, Staph. cohnii, Staph. gallinarum, Staph. hominis, Staph. hyicus,* and *Staph. succinus*, while *Staph. succinus* was the only species unique to BP.

Overall, quarter-level prevalence of IMI by pathogen was similar between BP and TS in the current study. BP systems have a number of advantages, including a smaller initial investment when compared to a new FS or TS barn (Barberg et al., 2007a; Janni et al., 2007), although the cost year-over-year for bedding is substantial (Shane et al., 2010). BP are designed for cow comfort (Barberg et al., 2007b; Bewley et al., 2012), and prevalence of lameness, foot, and leg injuries in these systems has been found to be less than TS and FS (Barberg et al., 2007b). For producers considering a transition from outdated TS barns, BP may be a viable option for dairy cattle housing in the Northeastern US. However, more research is needed in order to compare these facility types with sufficient statistical power to account for herd-level effects.

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| Table 1. Quarter-level prevalence of pathogens (or grouping of similar pathogens) causing intramammary infections [median (range)] by farm, stratified by facility type. 3,332 quarter-level observations were collected from 1,456 quarters belonging to 382 cows during a longitudinal, cross-sectional observational study of 10 certified organic dairy farms in Vermont (US). The quarter-level prevalence represents the percent of sampled quarters infected with a particular pathogen over all sampled quarters at risk where IMI status could be determined for that farm visit. Median and range of quarter-level prevalence for each herd were calculated using all consecutive visits to a particular farm. Median and range of quarter-level prevalence for tiestalls (TS) and bedded packs (BP) were calculated over all visits to TS (n = 15) and BP (n = 15), respectively. Overall median and range of quarter-level prevalence were calculated using all visits to all 10 farms (n = 30). | | | | | | | | | | | | | | | |
|  | | | TS-1 | TS-2 | TS-3 | TS-4 | TS-5 | TS avg. | BP-1 | BP-2 | BP-3 | BP-4 | BP-5 | BP avg. | Overall |
| Num. farm visits | | | 3 | 3 | 3 | 3 | 3 | 15 | 3 | 4 | 3 | 2 | 3 | 15 | 30 |
| Pathogen (group) | | |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | *Staphylococcus aureus* | | 3.5 (3.1-4) | 1.9 (1.7-2.4) | 4 (3.4-4.1) | 3.5 (3.2-3.9) | 0.9 (0.9-1) | 3.2 (0.9-4.1) | 4.8 (4.7-8.4) | 13.1 (11.7-14.1) | 0.8 (0-2.6) | 1.3 (0.9-1.7) | 0.8 (0-0.8) | 2.6 (0-14.1) | 3.2 (0-14.1) |
|  | Non-*aureus* staphylococci and mammaliicocci | | 10 (9.7-10.4) | 19.6 (16.7-23.2) | 24 (22.3-25.6) | 15.8 (14.2-18.5) | 20.4 (19-24.5) | 19 (9.7-25.6) | 24.8 (21.9-39.8) | 14.1 (12.8-18.2) | 23.6 (21.9-25.5) | 19.4 (17.6-21.1) | 20.6 (19.4-22.4) | 21.1 (12.8-39.8) | 20 (9.7-39.8) |
|  | | *Staphylococcus agnetis* | 2 (1.8-2.1) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0.9) | 0 (0-2.1) | 2.4 (0.8-3.6) | 1.1 (0-2) | 0 (0-0) | 0.9 (0.8-0.9) | 0.8 (0.8-0.9) | 0.8 (0-3.6) | 0.8 (0-3.6) |
|  | | *Staphylococcus auricularis* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) |
|  | | *Staphylococcus capitis* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0.8 (0-0.8) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) |
|  | | *Staphylococcus chromogenes* | 6 (5.3-6.3) | 13.1 (11.7-15.9) | 16.2 (14.9-16.8) | 8.8 (7.1-8.9) | 15.5 (13.8-15.5) | 13.1 (5.3-16.8) | 15.2 (13.3-21.7) | 7.8 (6.5-10.1) | 19.5 (17.5-21.8) | 15.9 (14.3-17.4) | 16.8 (16.1-17.6) | 16.1 (6.5-21.8) | 14.6 (5.3-21.8) |
|  | | *Staphylococcus cohnii* | 0 (0-0) | 0.9 (0.8-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) |
|  | | *Staphylococcus devriesei* | 0 (0-0) | 0.9 (0.8-1.2) | 0 (0-0) | 0 (0-0) | 1.9 (1.7-2.7) | 0 (0-2.7) | 0.8 (0.8-2.4) | 1 (0-1.1) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-2.4) | 0 (0-2.7) |
|  | | *Staphylococcus epidermidis* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0 (0-1) |
|  | | *Staphylococcus equorum* | 0 (0-0) | 3.7 (1.7-3.7) | 0 (0-0) | 0 (0-0) | 1 (0.9-1.7) | 0 (0-3.7) | 0 (0-1.2) | 0 (0-1.1) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-3.7) |
|  | | *Staphylococcus gallinarum* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0.8 (0-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-1.2) |
|  | | *Staphylococcus haemolyticus* | 1 (0.9-1) | 0 (0-0) | 0.8 (0.8-1.7) | 1.6 (1.6-1.8) | 0 (0-0.9) | 0.9 (0-1.8) | 3.1 (2.4-4.8) | 3 (2.1-3.3) | 3.3 (2.7-3.5) | 0.9 (0.8-0.9) | 0.8 (0-1.9) | 2.7 (0-4.8) | 1.3 (0-4.8) |
|  | | *Staphylococcus hominis* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) |
|  | | *Staphylococcus hyicus* | 0 (0-0) | 0 (0-0) | 0.8 (0.8-0.9) | 0 (0-0) | 0 (0-0) | 0 (0-0.9) | 0 (0-0) | 1 (0-1.1) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.1) | 0 (0-1.1) |
|  | | *Staphylococcus pseudintermedius* | 0 (0-0) | 0.9 (0.8-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) |
|  | | *Staphylococcus saprophyticus* | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) |
|  | | *Staphylococcus simulans* | 1 (0.9-1) | 0 (0-0) | 3.2 (2.5-4.3) | 3.2 (3.1-3.5) | 0 (0-0) | 1 (0-4.3) | 2.4 (2.3-2.4) | 0 (0-0) | 0.9 (0.8-0.9) | 0.9 (0.8-0.9) | 1.5 (0.8-1.9) | 0.9 (0-2.4) | 0.9 (0-4.3) |
|  | | *Staphylococcus succinus* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1) | 0 (0-1) |
|  | | *Staphylococcus warneri* | 0 (0-0) | 0 (0-0) | 2.5 (2.4-2.6) | 1.6 (1.6-1.8) | 0 (0-0) | 0 (0-2.6) | 1.6 (0.8-2.4) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-2.4) | 0 (0-2.6) |
|  | | *Staphylococcus xylosus* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 1 (0.9-1.8) | 0 (0-1.8) | 0 (0-0) | 0.5 (0-1.1) | 0 (0-0) | 0.9 (0.8-0.9) | 0.8 (0.8-0.9) | 0 (0-1.1) | 0 (0-1.8) |
|  | | *Mammaliicoccus fleurettii* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 1 (0-1.8) | 0 (0-1.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.8) |
|  | | *Mammaliicoccus sciuri* | 0 (0-0.9) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0.9) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.9) |
|  | | *Mammaliicoccus vitilinus* | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.9) | 0 (0-0.9) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.9) |
|  | *Streptococcus dysgalactiae* | | 0 (0-0) | 3.3 (2.8-7.3) | 1.7 (0.8-1.7) | 0.8 (0.8-0.9) | 0 (0-0) | 0.8 (0-7.3) | 0 (0-0) | 1.6 (1.1-2) | 0.9 (0-1.8) | 0 (0-0) | 0 (0-0) | 0 (0-2) | 0.4 (0-7.3) |
|  | *Streptococcus uberis* | | 1 (0.9-1) | 5.6 (5-6.1) | 5 (4-5.1) | 2.4 (2.4-2.6) | 0.9 (0.9-1) | 2.4 (0.9-6.1) | 3.6 (3.2-5.5) | 4.2 (1.1-6.1) | 2.4 (1.8-3.5) | 0.4 (0-0.8) | 5.3 (4-5.6) | 3.6 (0-6.1) | 3.4 (0-6.1) |
|  | *Aerococcus* spp.1 | | 1 (0-1.8) | 0 (0-1.7) | 0 (0-1.7) | 0 (0-1.6) | 0 (0-0.9) | 0 (0-1.8) | 0 (0-4.8) | 0.5 (0-4) | 0 (0-0.9) | 0 (0-0) | 1.6 (0-3.7) | 0 (0-4.8) | 0 (0-4.8) |
|  | Other streptococcal and streptococcal-like organisms2 | | 0 (0-0) | 0 (0-0.8) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-1.2) | 0.5 (0-1.1) | 0 (0-1.6) | 0 (0-0) | 0.8 (0-1.6) | 0 (0-1.6) | 0 (0-1.6) |
|  | *Corynebacterium* spp.3 | | 4 (0.9-6.3) | 7.5 (6.7-11) | 2.4 (0.8-2.6) | 1.6 (0-1.6) | 0 (0-0.9) | 1.6 (0-11) | 0.8 (0-8.4) | 11.5 (5.3-20.2) | 0 (0-0) | 0.4 (0-0.8) | 0 (0-0) | 0 (0-20.2) | 0.9 (0-20.2) |
|  | *Kocuria* spp.4 | | 1 (0-3.5) | 0 (0-0.8) | 1.6 (0-2.6) | 0 (0-1.6) | 0 (0-0.9) | 0 (0-3.5) | 0 (0-1.6) | 0 (0-0) | 0 (0-2.6) | 0 (0-0) | 0 (0-0) | 0 (0-2.6) | 0 (0-3.5) |
|  | Other gram-positive bacteria | | 1 (0.9-1) | 0 (0-1.2) | 0.8 (0-0.8) | 0.8 (0-1.6) | 0 (0-1.8) | 0.8 (0-1.8) | 0.8 (0-0.8) | 2.1 (1-4.3) | 0 (0-0) | 1.3 (0.8-1.8) | 0.8 (0-0.8) | 0.8 (0-4.3) | 0.8 (0-4.3) |
|  | Coliforms5 | | 0 (0-0) | 0 (0-0.9) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.9) | 0 (0-0) | 1.1 (1-2) | 1.8 (0-2.4) | 0.5 (0-0.9) | 0 (0-0) | 0 (0-2.4) | 0 (0-2.4) |
|  | Other gram-negative bacteria | | 0.9 (0-2) | 0.9 (0.8-1.2) | 0 (0-0) | 0.8 (0-0.8) | 0.9 (0-0.9) | 0.8 (0-2) | 0 (0-0) | 0.5 (0-2) | 0 (0-0.9) | 0.8 (0-1.7) | 0 (0-0.9) | 0 (0-2) | 0 (0-2) |
|  | *Candida rugosa* | | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-1.2) | 0 (0-1.2) |
|  | Unable to be identified | | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0) | 0 (0-0.8) |
| 1 *Aerococcus* sp. (genus-level identification only), *Aerococcus viridans* | | | | | | | | | | | | | | | |
| 2Other streptococcal and streptococcal-like organisms not listed separately: *Streptococcus* sp*.* (genus-level identification only)*, Streptococcus canis, Enterococcus saccharolyticus* | | | | | | | | | | | | | | | |
| 3 *Corynebacterium* sp. (genus-level identification only), *C. amycolatum, C. callunae, C. casei, C. confusum, C. glutamicum, C. stationis, C. ulcerans, C. variabile, C. xerosis* | | | | | | | | | | | | | | | |
| 4 *Kocuria* sp. (genus-level identification only), *Kocuria* *carniphila, Kocuria* *palustris* | | | | | | | | | | | | | | | |
| 5 *Enterobacter* sp. (genus-level identification only), *Escherichia coli, Klebsiella aerogenes, Klebsiella pneumoniae, Klebsiella variicola, Serratia marcescens* | | | | | | | | | | | | | | | |

***Declarations***

*Ethics approval and consent to participate*

STROBE-VET (Strengthening the Reporting of Observational Studies in Epidemiology–Veterinary Extension) statement guidelines were followed in the reporting of this study (O'Connor et al., 2016). Animal use for this project was approved by the University of Vermont Institutional Animal Care and Use Committee (IACUC; protocol #19-001).

*Consent for publication*

Not applicable

*Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors have not stated any conflicts of interest.

*Funding*

This project was funded by the Organic Agriculture Research and Extension Initiative (OREI) from the National Institute of Food and Agriculture (USDA-NIFA grant 2018-51300-28561). The first author, Caitlin E. Jeffrey was supported by a USDA-NIFA Predoctoral Fellowship award (grant 2022-67011-36567).

*Authors' contributions*

Caitlin Jeffrey conceptualized the study, acquired funding, coordinated farm recruitment and sampling, conducted on-farm sample collection, managed and curated the data, conducted the data analysis, prepared data visualizations and presentation, wrote the original and final drafts. Pamela Adkins, conducted isolate species identification by MALDI-TOF. John Barlow conceptualized the study, acquired funding, supervised the research, conducted on-farm sample collection, reviewed and edited the manuscript.

*Acknowledgements*

The authors thank the organic dairy producers who agreed to participate in this study, for giving us their time and allowing us to collect samples from their farms. We are grateful to the numerous University of Vermont undergraduate students who assisted with sample collection. We thank the laboratory staff at the Vermont State Agricultural and Environment Laboratory for determination of somatic cell counts from the quarter milk samples. We thank Paige Isensee, Natalie Sexton, Madyson Marrs, and Allena Radford, who provided laboratory assistance in the Adkins lab at the University of Missouri.

*Authors' information*

Not applicable

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