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Intramammary infections in lactating Jersey cows: Prevalence of microbial organisms and association with milk somatic cell count and persistence of infection

Samantha R. Haw,[1] P. R. F. Adkins,1* V. Bernier Gosselin,[2] S. E. Poock,[1] and J. R. Middleton[1]
[1] Department of Veterinary Medicine and Surgery, University of Missouri, Columbia 65211
[2] Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland 3012

ABSTRACT

There is limited data available regarding pathogens causing intramammary infections (IMI) in Jersey cows. The objectives of this study were to characterize the prevalence of IMI caused by different microorganisms in lactating Jersey cattle and evaluate the associations among microbes and somatic cell count (SCC) and persistence of IMI.

This prospective, observational, longitudinal study included lactating Jersey cows (n = 753) from 4 farms within a 250-mile radius of Columbia, Missouri. Quarter foremilk samples were aseptically collected monthly for 3 consecutive months. Microorganisms were identified using aerobic milk culture and MALDI-TOF mass spectrometry. A commercial laboratory measured SCC using flow cytometry. Milk culture results were used to classify single microorganism infections as persistent (same microorganism species identified at first sampling and one other sampling) or non-persistent infection. Mixed models were built to evaluate the associations between IMI status and lnSCC as well as persistence and lnSCC.

Overall, staphylococci were the most commonly isolated microorganisms among the 7,370 quarter-level milk samples collected. Median prevalence (using all 3 samplings) of specific microbes varied among farms; however, *Staphylococcus chromogenes* was a common species found at all farms. The most common microbial species that persisted were *Staph. chromogenes, Staphylococcus aureus, Staphylococcus simulans,* and *Streptococcus uberis. Streptococcus dysgalactiae* and *Staph. aureus* were the IMI associated with the most inflammation based on lnSCC. The small number of herds included in this study with the large variation in herd type limits the generalizability of the data. However, results of this study seem to be similar to those of previous studies in other breeds, suggesting management factors are more important than breed-specific differences when evaluating causes of IMI and associated subclinical mastitis.

KEYWORDS: Subclinical mastitis, somatic cell count, intramammary infection, Jersey, bovine

INTRODUCTION

Jersey cattle make an important contribution to the US dairy economy as the second most prevalent breed (Council on Dairy Cattle Breeding, 2021). Jersey milk contains higher concentrations of fat, protein, calcium, and magnesium when compared with that of Holstein, Brown Swiss, Simmental, and Alpine Grey cows (Manuelian et al., 2018). The uniquely nutrient-dense composition of Jersey milk makes it ideally suited for incorporation into cheese, which currently accounts for the largest share of per capita US dairy consumption (USDA Economic Research Service, 2021). Beyond milk composition, Jerseys offer the benefits of a more compact body frame, greater feed efficiency, lower consumption of natural resources, reduced carbon footprint, and lower production of waste (Capper and Cady, 2012, Gonzalez-Peña et al., 2020). These factors combined have likely contributed to the growth of the Jersey cattle population within the US dairy herd, increasing from 7.3% in 2016 to 12.7% in 2020 among cows enrolled in DHIA with breed records (Council on Dairy Cattle Breeding, 2021).

While the Jersey breed may offer distinct advantages over the Holstein breed, breed-dependent genetic differences are known to impact disease resistance and need to be considered (Kelm et al., 2001). However, few studies have evaluated the association between breed and IMI or mastitis associated pathogens. Previous work has identified that some breed variation can exist when characterizing NAS species from dairy cattle. For example, *Staphylococcus haemolyticus* is more common in quarter milk samples from Swedish Holsteins

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*Corresponding author: Pamela R. F. Adkins, 315 Clydesdale Hall, 900 East Campus Drive, Columbia, Missouri USA 65211, (573) 882-6857, adkinsp@missouri.edu

compared with Swedish Reds (Nyman et al., 2018) and *Staphylococcus devriesei* was more common on the teat ends of red and white Holsteins cows compared with black and white Holstein cows (De Visscher et al., 2016). Conversely, another study identified no impact of breed when evaluating pathogens associated with clinical mastitis among Swedish dairy cows (Duse et al., 2021).

When evaluating Jersey cattle specific breed differences, Jersey cows have been reported to have a lower prevalence of subclinical mastitis (Youngerman et al., 2004) and clinical mastitis (Berry et al., 2007) than Holstein cows. However, Jersey cows have also been reported to have a higher milk SCC compared with Holstein cows based upon test-day records (Sewalem et al., 2006) and when using composite milk samples (Sewalem et al., 2006, Berry et al., 2007). Elucidating the reason behind these findings is difficult, as most of the existing studies in which subclinical mastitis pathogen-specific effects were explored have failed to explain breed-associated differences in SCC (Valckenier et al., 2019, Martins et al., 2020). It is unclear if these breed differences in SCC are attributable to variations in the immune response to IMI with different microbes or are associated with the lower quantity of milk produced by Jersey cattle relative to Holsteins (Prendiville et al., 2010). The majority of subclinical mastitis research has been conducted in Holstein cattle or other non-Jersey breeds (Bobbo et al., 2017, Heikkila et al., 2018, Thorberg et al., 2009). Likewise, investigations of chronic subclinical mastitis, have been predominantly Holstein focused (Gonçalves et al., 2020, Martins et al., 2020). Thus, further work is needed to investigate subclinical mastitis in Jerseys as well as how specific microbial pathogens potentially influence SCC.

Given the increasing number of Jersey cattle in the US and the potential breed-specific advantages they offer in dairy farming and food production, a need exists to further understand the factors that impact udder health and milk production in Jersey cows. The objectives of this study were to characterize the prevalence of IMI caused by different microorganisms in lactating Jersey cows and evaluate organism-specific associations with SCC and persistence of IMI.

MATERIALS AND METHODS

Herds

A prospective, observational, longitudinal study was conducted on 4 all-Jersey cow dairy farms within a 250-mile radius of Columbia, Missouri. Herds were recruited on a voluntary basis. All herds were enrolled in DHIA. Individual cow data consisting of DIM and parity were retrieved from DHIA records. The study protocol (#9896) was approved by the University of Missouri Animal Care and Use Committee.

Milk Sample Collection

Individual mammary gland (quarter) foremilk samples were collected from all functional quarters of all lactating cows once monthly for 3 consecutive months. Herd enrollment dates ranged from September 2020 through January 2021. For all herds that utilized a milking parlor (n = 3), samples were collected during routine milking. Farm personnel disinfected and dried teats according to each farm's established pre-milking hygiene protocol. Samples were then collected as described below by the study authors (SRH and PRFA) and research assistants, who had received in-person training and supervised practice to ensure proper, consistent technique. Sample collection was performed while wearing disposable nitrile gloves. For the single herd that used an automated milking system (**AMS**), cows were sampled between milkings and the teats were prepared for sampling by the research personnel using the National Mastitis Council recommended protocol (Middleton et al., 2017). Briefly, teat preparation included brushing loose dirt and debris from the gland and teats. Next, forestripping was completed and then an iodine-based teat disinfectant was applied to each teat and allowed 30 s of contact time. Teats were then dried with individual paper towels. After completion of the pre-milking hygiene protocol on all herds, the apex and barrel of each teat were scrubbed with a 70% isopropyl alcohol-soaked non-woven cotton gauze immediately before sample collection. Each quarter was manually stripped of a few streams of milk before aseptically collecting milk into a sterile plastic vial for standard aerobic culture (Middleton et al., 2017). Immediately after aseptic collection, milk from each quarter was collected into separate nonsterile vials containing a 2-bromo-2-nitropropane-1, 3-diol preservative tablet (Broad Spectrum Microtabs II, D & F Control Systems, Inc., Dublin, CA, USA) for SCC enumeration. All samples were numbered and labeled by quarter, and tube numbers were correlated to individual cow identity. Samples were stored on ice and transported to the laboratory. Milk samples for culture were frozen (−20°C) within 8 h of collection. Milk samples for SCC were stored at room temperature (~22°C) and shipped to Mid-South Dairy Records (Springfield, MO, USA) for analysis the following day.

Microorganism Isolation and Identification

Frozen milk samples were stored for 1–25 d before culture. Samples were thawed at room temperature (~22°C) and vortex-mixed before being plated. Using a disposable, sterile cotton-tipped applicator, approximately 10 μL of each milk sample were spread onto half of a Columbia blood agar plate with 5% sheep blood (Remel, Lenexa, KS, USA) for aerobic culture. Plates were incubated at 37°C and assessed for growth at 24 and 48 h. Colony counts were recorded and colony morphological characteristics including color, size, and hemolysis were documented according to the National Mastitis Council guidelines (Middleton et al., 2017). Milk samples yielding >2 colony types were considered contaminated.

All microbial isolates, except those from contaminated plates, were sub-cultured and MALDI-TOF MS was performed to identify microorganism genus and, when possible, species. The plate extraction method was used for MALDI-TOF identification in which 1–2 isolated colonies were applied to a MALDI-TOF target plate (Bruker Daltonics, Billerica, MA, USA) in duplicate and covered with 0.7 μL of 70% formic acid. The target spots were air-dried, covered with 0.7 μL MALDI matrix solution (Bruker Daltonics), and then air-dried again. The target plate was calibrated using the *Escherichia coli* Bacterial Test Standard (Bruker Daltonics) in duplicate. Isolate mass spectral analysis was captured using a MALDI-TOF mass spectrometer (Microflex LT, Bruker Daltonics) and each sample was assigned an analysis score determined by the similarity of the isolate's spectrum to the manufacturer's reference database and the University of Missouri Udder Health Laboratory custom database (Adkins et al., 2018) using the internal software (flexAnalysis, Bruker Daltonics).

Species-level microorganism identification was considered reliable when a MALDI-TOF analysis score on at least 1 of the duplicates was ≥2.0 for all non-*Staphylococcus* spp. and considered reliable for all *Staphylococcus* spp. and *Mammaliicoccus sciuri* when a score of ≥1.7 was obtained (Cameron et al., 2017, Cameron et al., 2018). Non-*Staphylococcus* spp. scores between 1.7 and 1.99 resulted in only genus-level identification. If an isolate analysis score was <1.7, the isolate was re-cultured and re-analyzed in duplicate. Isolates with scores still below this threshold were classified as unidentified.

Definitions

Definitions of IMI were based on an assumption of inoculum size of ~10 μL and adapted from Dohoo et al., 2011. A quarter was defined as having an IMI if >1 colony (Dohoo et al., 2011) was isolated on Columbia blood agar for all microorganisms except *Staphylococcus aureus* and *Streptococcus agalactiae*, where an IMI was defined based on isolation of ≥1 colony (Dohoo et al., 2011), or for *Bacillus* spp. when ≥5 colonies (Rowe et al., 2019) were isolated. Samples were categorized as having no significant growth if no colonies were present on Columbia blood agar after 48 h or if colony counts were below the stated definition for IMI.

Quarter milk samples yielding 2 distinct microorganisms were defined as mixed infections. For the purpose of describing overall occurrence and prevalence, mixed infections were grouped together and not further defined. Samples that yielded a culture result but whose isolates could not be identified at genus or species level by MALDI-TOF were categorized as unidentified in the descriptive analysis. Missing samples, blind quarters, unidentified microorganisms, mixed infections, and contaminated samples were denoted as missing data (manually censored) in the evaluation of persistence of IMI and the statistical modeling.

For the evaluation of IMI persistence, quarters not sampled at the first visit and quarters only sampled at first visit were excluded from analyses. Quarters yielding a censored result at first visit were also excluded. An IMI was considered persistent when the same microorganism, identified to the species level, was isolated at first sampling and at least 1 other sampling. Accordingly, quarters in which one of the second or third sampling outcomes yielded the same microorganism as the first sampling, but no IMI or censored result at the remaining sampling, were considered persistent. Intramammary infections were not classified as persistent if first sampling yielded a single species-level microorganism that was not subsequently encountered at another visit. Intramammary infections with censored result at the second or third sampling and that could not be classified as persistent, as defined above, were excluded from analysis.

SCC Enumeration

Samples for SCC were shipped to a commercial laboratory (Mid-South Dairy Records, Springfield, MO, USA). Somatic cell count enumeration was determined using an automated flow cytometric counter (Bentley Somacount FCM, Bentley Instruments, Chaska, MN, USA) and all results reported to the corresponding author (PRFA) by e-mail.

Statistical Analyses

Using the definitions above, absolute numbers were determined for all possible outcomes across all sam-

plings within herd. Median prevalence of each outcome was calculated using data from all 3 visits to each farm (Table 2). Persistent infections were also tabulated (Table 5).

Statistical models were constructed to evaluate the association between microorganism-specific IMI and milk SCC. The SCC natural logarithm (**lnSCC**) was used for statistical analyses.

Somatic cell count

To improve models convergence, the list of quarter-level outcomes was narrowed to known mastitis-causing microorganisms that occurred more than 10 times in the overall data set. Categories included microbial species-level data for the most frequently encountered microorganisms. Relevant microorganism genera and species encountered fewer than 10 times in the overall data set were grouped with similar microorganisms for statistical evaluation (for example all other streptococcal and streptococcal-like organisms [**SSLO**] excluding *Streptococcus dysgalactiae* and *Streptococcus uberis* were clustered into 1 group). *Corynebacterium amycolatum* was grouped with other *Corynebacterium* species, despite its high frequency of occurrence, due to it being considered a minor species with similar behavior to other *Corynebacterium* spp. (Gonçalves et al., 2016). A classification group of "Other NASM" included non-*aureus* staphylococcal and mammaliicoccal species (**NASM**) identified at a frequency too low to be assessed independently by species. No significant growth was included as a negative comparator group. A linear mixed model was constructed to evaluate the association of IMI status (with no growth as reference) and lnSCC with random effects for quarter nested within cow nested within farm. Data, subsequently referred to as "observations," were censored when there was missing information at the cow-level (including parity and DIM) or at the quarter level (including undetermined SCC or if IMI status was unidentified, mixed IMI, or contaminated). Robust estimation of standard errors was used. Unconditional relationships between lnSCC and IMI status, parity (1, 2, 3, 4, ≥ 5), and DIM (including polynomial terms) were individually evaluated in reduced models for inclusion in the multivariable model. Associations among independent variables were assessed, as were interactions in reduced models. Statistically significant interactions ($P < 0.05$) were evaluated in the model. Normality and homoscedasticity of residuals were visually assessed. Sidak adjustment was used for multiple comparisons.

TABLE 1. Sample population herd descriptive characteristics including management and production information in addition to number of cows (and quarters) sampled and bulk tank SCC for each consecutive monthly herd visit. The study enrolled 4 all Jersey herds, including a total of 753 cows, within 250 miles of Columbia, Missouri. Quarter milk samples were collected for 3 consecutive months from all lactating cows

	HOUSING STYLE	MILKING FACILITIES	AVERAGE ANNUAL MILK PRODUCTION (kg)	COWS[1] (QUARTERS) SAMPLED AND BULK TANK SCC[2] (cells/mL)			Number used[3]	Sampled quarter-level SCC ($\times 10^3$ cells/ml)		
				VISIT 1	VISIT 2	VISIT 3		Median	Q1-Q3[4]	Range
Herd 1	Confinement with sand-bedded free stall	Parlor, double 10 herringbone	7,394	246 (969) 227,500	250 (973) 232,000	240 (933) 148,000	2860	18	8–63	2–9999
Herd 2	Dry lot and pasture	Parlor, double 5 herringbone	6,932	110 (430) 388,000	105 (411) 233,000	105 (413) 315,000	1251	53	16–211	2–9999
Herd 3	Pasture and loose housing	Parlor, double 4 herringbone	6,030	80 (312) 340,000	75 (293) 385,000	77 (286) 680,000	776	60	26–161	2–9999
Herd 4	Confinement with sand-bedded free stall	Automatic milking system (AMS)	12,161	207 (808) 150,000	196 (756) 160,000	200 (786) 140,000	2329	33	12–123	2–9999

[1]Number represents lactating population.
[2]Bulk Tank SCC numbers used were obtained for the herd manager and were collected around the time of each herd visit.
[3]After exclusion of samples due to lack of SCC results (sample lost, cow ID not recorded).
[4]First and third quartile.

IMI persistence

Using data from infected quarters at the first sampling, the list of quarter-level outcomes was narrowed to microorganisms that occurred ≥5 times in the overall data set. In this data set, *Staphylococcus haemolyticus*, *Staphylococcus gallinarum*, and *Staphylococcus xylosus* were grouped with other NASM. *Serratia marcescens* was grouped with other coliforms. A mixed logistic regression model was built to evaluate the association between persistence status (dependent variable) and species-level IMI status, with random effects at the cow and farm levels. *Staphylococcus chromogenes* was selected as reference IMI status due to its high prevalence and known tendency to cause persistent IMI. Unconditional relationships between persistence and lnSCC, IMI species group (*Staph. chromogenes, Staphylococcus simulans, Staph. aureus,* other NASM, *Corynebacterium* spp., *Strep. dysgalactiae*, *Strep. uberis*, SSLO, *Candida* spp., and coliforms), parity (as above), and DIM were individually evaluated in reduced models before inclusion in the multivariable model. Associations among independent variables were assessed. Sidak adjustment was used for multiple comparisons. Analyses were conducted using STATA version 16.1 (StataCorp LLC, College Station, TX).

RESULTS

Herd size ranged from 92 to 280 lactating cows. Table 1 describes herd characteristics including herd size, housing style, milking facilities, average annual milk production per cow, number of animals and quarters sampled per visit, and bulk tank SCC at each sampling period. Enrolled herds included a mixture of indoor confinement and outdoor housing. Three herds had milking parlors and 1 utilized AMS. A total of 753 cows, yielding 2,996 viable quarters, were enrolled in the study; 7,370 quarter-level milk samples were collected over the 3 sampling periods. Sixteen quarters were not sampled due to being blind or injured. Among the 753 cows, 493 (65%) cows had samples collected at all 3 samplings, 149 (20%) cows had samples collected

TABLE 2. Median (range) quarter-level microorganism prevalence calculated within each herd using all 3 consecutive monthly milk samples. MALDI-TOF MS results reported as a percentage and organized in descending order of prevalence by the most frequently encountered species or grouping of similar microorganisms. The study enrolled 4 all Jersey herds, including a total of 753 cows, within 250 miles of Columbia, Missouri.

Diagnosis	ALL %	Herd 1%	Herd 2%	Herd 3%	Herd 4%
No Significant Growth	75.2	86.3 (82.9–88.5)	76.7 (68.8–80.8)	70.3 (64.7–72)	64.9 (59.3–68.7)
Specific Infections	12.8	6.8	13.6	17.2	18
Staphylococcus chromogenes	4.3	2.5 (2.4–2.6)	5.6 (4.6–5.6)	6.1 (5.4–7)	5 (4.8–6.6)
Staphylococcus simulans	2.7	0 (0–0)	0 (0–0.2)	0 (0–0)	8.7 (8–8.9)
Staphylococcus aureus	1.2	0.2 (0.1–0.3)	0.7 (0.5–2.3)	6.8 (6.7–8)	0.1 (0–0.1)
Streptococcus dysgalactiae	0.5	0.7 (0.3–0.7)	0 (0–0.5)	0.7 (0.3–1.7)	0.2 (0.1–0.8)
Streptococcus uberis	0.3	0.5 (0.3–0.6)	0.5 (0.5–0.7)	0 (0–0.3)	0 (0–0)
Staphylococcus haemolyticus	0.3	0 (0–0)	0.9 (0.5–1)	0 (0–0)	0.4 (0.3–0.5)
Staphylococcus gallinarum	0.2	0.2 (0.1–0.4)	0.2 (0.2–0.5)	0 (0–0)	0.2 (0.1–0.4)
Serratia marcescens	0.2	0.1 (0.1–0.2)	0.5 (0.2–1.9)	0 (0–0.3)	0 (0–0)
Staphylococcus xylosus	0.2	0 (0–0)	0.2 (0.2–0.2)	0 (0–0)	0.4 (0.4–0.4)
Corynebacterium spp.[1]	0.8	0.1 (0.1–0.6)	1.4 (1–1.7)	0.3 (0–0.7)	0.9 (0.7–2.2)
Other SSLO [2]	0.7	1.1 (0.9–1.4)	0.5 (0–0.5)	0.7 (0.6–1)	0.4 (0.3–0.4)
Candida spp.[3]	0.3	0.2 (0.2–0.8)	0.5 (0.2–1.2)	0 (0–0.3)	0 (0–0)
Bacillus spp.[4]	0.1	0.4 (0–0.4)	0 (0–0.2)	0 (0–0)	0 (0–0)
Coliforms[5]	0.3	0.1 (0–0.1)	0.2 (0.1–1)	0 (0–0.3)	0.1 (0.1–0.5)
Other NASM[6]	0.3	0.1 (0–0.4)	0.2 (0–0.5)	0.3 (0–0.3)	0.4 (0.1–0.6)
Undetermined Identity	1.1	0.8 (0.7–1.8)	1.5 (0.7–1.9)	2 (1.4–2.2)	0.9 (0.6–0.9)
Mixed Infection	0.8	0.5 (0.4–0.6)	1.2 (0.5–1.6)	1.4 (0–2)	0.8 (0.5–1.4)
Contaminated	10.1	6.5 (3.4–7.1)	4.1 (4–17.7)	7.8 (5.9–18.3)	17.2 (8.9–22.2)

[1]*Corynebacterium* spp.*, *Corynebacterium amycolatum, Corynebacterium ulcerans, Corynebacterium xerosis*..

[2]Includes other streptococcal and streptococcal-like organisms (SSLO) - *Streptococcus* spp.*, *Streptococcus canis, Streptococcus gallolyticus, Streptococcus lutetiensis,* strep-like species (*Aerococcus* spp.*, *Aerococcus viridans, Enterococcus faecalis, Enterococcus faecium, Lactococcus* spp.*, *Lactococcus garvieae, Lactococcus lactis*), excluding *Strep.* listed in table above.

[3]*Candida* spp.*, *Candida kefyr, Candida krusei, Candida lusitaniae, Candida rugosa, Candida tropicalis*..

[4]*Bacillus* spp.*, *Bacillus licheniformis, Bacillus sonorensis*..

[5]Includes coliform and other gram-negative bacteria (*Enterobacter xiangfangensis, Escherichia coli, Proteus hauseri, Proteus vulgaris, Pseudomonas aeruginosa, Serratia ureilytica*), excluding *Serratia marcescens*..

[6]Includes other non-*aureus* staphylococcal and mammaliicoccal (NASM) species - *Staphylococcus agnetis/hyicus, Staphylococcus auricularis, Staphylococcus devriesei, Staphylococcus epidermidis, Staphylococcus equorum, Staphylococcus nepalensis, Mammaliicoccus sciuri, Staphylococcus warneri*, not listed in table above.

* Genus-level identification only.

at 2 samplings, and 111 (15%) cows had samples collected only once.

Overall, no significant growth was the most frequent outcome for quarter-level milk culture results (5,543/7,370; 75.2%). A total of 75 different microorganisms were identified to the genus and/or species level in this study. The most common microorganisms identified at first sampling were *Staph. chromogenes, Staph. simulans*, and *Staph. aureus*. *Staphylococcus chromogenes, Staph. aureus,* and *Strep. dysgalactiae* were identified on all 4 farms. Overall, NASM were among the most frequently identified microorganisms. *Staphylococcus chromogenes* was the most common microorganism (320/7,370; 4.3%) isolated overall but not always the most common microorganism found within herd, being the most common in half (2/4) of the sampled herds. *Staphylococcus aureus* and *Staph. simulans* were the most common microorganism identified in 1 herd each. *Corynebacterium amycolatum* was the fourth-most common microorganism (36/7,370; 0.5%) identified at the species level. *Streptococcus agalactiae* was isolated once. A large number of microorganisms (n = 63) were infrequently isolated (fewer than 15 times overall). The median prevalence of quarter-level outcomes calculated using all 3 samplings for each herd are reported in Table 2. The prevalence of each outcome varied slightly among farms. Overall, 13 different staphylococcal and 1 mammaliicoccal species were identified (Table 3). *Staphylococcus chromogenes* was present and highly prevalent among recovered staphylococcal and mammaliicoccal species identified on all farms. Despite many species being present, the majority of staphylococcal and mammaliicoccal species recovered from each herd were that of only 1–2 species and most other species of this group were identified relatively rarely.

Observations were omitted from further statistical analyses due to absence of the following information: unidentified microorganism (n = 84), mixed infection (n = 60), or contaminated sample (n = 742). Additional observations were excluded due to the presence of microorganisms that were infrequently isolated (3 or fewer occurrences), including a single occurrence of *Strep. agalactiae* (n = 31). Finally, observations were excluded due to missing cow identity (n = 1), parity (n = 100), or SCC (n = 141). After exclusion of the aforementioned data, 6,211 observations from 2,794 quarters of 737 cows were available for inclusion in the linear mixed model. The associations of lnSCC with parity and polynomial terms of DIM were statistically significant in unconditional analyses, but no longer statistically significant in the final model. An interaction ($P < 0.01$) of lnSCC with DIM was observed for some IMI categories, including an increase in lnSCC estimate for each 30-DIM increment for *Staph. aureus* (+0.12) and decreased lnSCC estimate for each 30-DIM increment for *Strep. uberis* (−0.45), *Serratia marcescens* (−0.14), *Corynebacterium* spp. (−0.02), and *Candida* spp. (−0.11). Model-derived SCC associated with microorganism-specific IMI are shown in Table 4. However, the results should be interpreted with caution, as the assumption of normality of residuals was not met. Many species or groupings were associated with a predicted SCC less than 200,000 cells/mL. The highest predicted SCC were associated with IMI caused by *Staph. aureus* and *Strep. dysgalactiae*. The predicted SCC associated with *Staph. haemolyticus*, *Bacillus* spp., and coliforms were not statistically significantly different from quarters with no growth.

For the analysis of IMI persistence, only quarters with IMI at first sampling were eligible (n = 358). After exclusion of 127 IMI due to censored second or third sampling, 231 IMI were considered, among which 185 IMI were classified as persistent and 46 were classified as non-persistent IMI (Table 5). The most common microorganism species causing persistent IMI were *Staph. chromogenes* (78/185), *Staph. simulans* (50/185), and *Staph. aureus* (22/185). For the persistence model, 4 *Bacillus* spp. IMI and one observation with missing SCC data were excluded. The association of IMI persistence with parity was not statistically significant in unconditional analyses. Days in milk and lnSCC showed an association with IMI persistence of $P = 0.08$ and $P = 0.20$, respectively, in reduced models, but were not statistically significant ($P > 0.05$) in the final model including IMI species. Only IMI species were retained in the final model. Compared with IMI caused by reference species *Staph. chromogenes*, the odds of persistence of IMI caused by *Corynebacterium* spp. (OR 0.004, 95% CI 0.00003–0.59) and by the grouping of SSLO (OR 0.001, 95% CI 0.000002–1.01) were lower. After adjusting for multiple comparisons between microorganism groups, there were no statistically significant differences.

DISCUSSION

To the authors' knowledge, there have been no similar studies specifically focused on characterizing IMI and organism-specific associations with SCC in Jersey cows. The present investigation provides information about the prevalence of IMI caused by different microorganisms in lactating Jersey cattle and the association among individual microorganisms, SCC, and persistence of IMI.

Staphylococcal species were among the most frequently encountered microorganisms identified. These findings are similar to previous reports in other dairy

TABLE 3. Frequency of MALDI-TOF MS diagnosis of *Staphylococcus* spp. and *Mammaliicoccus* spp. IMI outcomes among 3 consecutive monthly quarter level milk samplings within 4 all Jersey herds with percent of each species per herd shown parenthetically. The study included a total of 753 cows, within 250 miles of Columbia, Missouri

Diagnosis	Herd 1	Herd 2	Herd 3	Herd 4
Staphylococcus chromogenes	71 (79.8)	66 (64.7)	55 (45.5)	128 (35.2)
Staphylococcus simulans	0 (0.0)	1 (1.0)	0 (0.0)	201 (55.3)
Staphylococcus aureus	6 (6.7)	15 (14.7)	64 (52.9)	2 (0.5)
Staphylococcus haemolyticus	0 (0.0)	10 (9.8)	0 (0.0)	9 (2.5)
Staphylococcus gallinarum	7 (7.9)	4 (3.9)	0 (0.0)	6 (1.9)
Staphylococcus xylosus	0 (0.0)	3 (2.9)	0 (0.0)	9 (2.4)
Mammaliicoccus sciuri	2 (2.2)	0 (0.0)	0 (0.0)	3 (0.8)
Staphylococcus agnetis/hyicus[1]	2 (2.2)	1 (1.0)	1 (0.8)	2 (0.5)
Staphylococcus devriesei	0 (0.0)	2 (2.0)	0 (0.0)	0 (0.0)
Staphylococcus epidermidis	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)
Staphylococcus auricularis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Staphylococcus equorum	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Staphylococcus nepalensis	1(1.1)	0 (0.0)	0 (0.0)	0 (0.0)
Staphylococcus warneri	0 (0.0)	0 (0.0)	1(0.8)	0 (0.0)
Total	89	102	121	364

[1]These species cannot be distinguished with MALDI-TOF MS.

cattle breeds where NASM are the most commonly isolated microorganisms from all quarter milk samples (Zigo et al., 2022) and of mastitic quarter samples (Heikkila et al., 2018) . *Staphylococcus chromogenes* and *Staph. simulans* were among the most commonly isolated microorganisms in this study. *Staphylococcus chromogenes* was found to be one of the most common microorganisms on all farms, which is similar to findings from other studies in non-Jersey breeds where all quarters, including clinical mastitis cases, were sampled 1–2 times monthly (Mørk et al., 2012) or predominantly once monthly (Piessens et al., 2011) from quarter milk samples of cows identified with subclinical mastitis following composite-sample-positive results for CNS (Tomazi et al., 2015). *Staphylococcus aureus* was the third most commonly detected microorganism (87/7,370; 1.2%), and although 1 farm had the majority of the *Staph. aureus* isolates, this microorganism was detected on all farms. *Staphylococcus aureus* is a major mastitis pathogen that often responds poorly to treatment, can persist in the infected quarter, and is associated with long-term loss in milk production (Heikkila et al., 2018). The immune response of Holstein and Jersey cows during a *Staph. aureus*-induced mastitis challenge has been evaluated, and it was determined that the magnitude, temporal onset, and duration of the innate immune response were highly conserved between the 2 breeds (Bannerman et al., 2008). Although there are genetic differences between these 2 cattle breeds, the host defense mechanisms are apparently highly conserved (Bannerman et al., 2008). Therefore, it is likely that management factors of these herds more significantly affect *Staph. aureus* prevalence and prevention than breed attributes, with respect to Holstein and Jersey cows. Although the focus of this study was not risk factor assessment, some differences in outcomes between farms were evident. For example, similar to the findings for *Staph. aureus*, most of the *Staph. simulans* were isolated from 1 farm. Interestingly, the farm with the high number of *Staph. simulans* IMI was an AMS herd. Specific risk factors for *Staph. simulans* IMI in AMS herds have not been reported.

The most common species that persisted were *Staph. chromogenes, Staph. simulans, Staph. aureus*, and *Strep. uberis*. Previous works have noted *Staph. chromogenes* to be more commonly associated with persistent IMI in comparison to other NASM species in subclinical mastitis quarters (Fry et al., 2014) and in quarter milk samples of heifers (Valckenier et al., 2020). In addition to *Staph. chromogenes, Staph. simulans* has also been reported to cause persistent IMI in subclinical mastitis quarters (Fry et al., 2014) and in quarter milk samples (Taponen et al., 2007). Similar to the present study, *Strep. uberis* IMI have been previously reported to persist, and subclinical infections were noted to have longer duration than clinical infections in quarter samples collected every 3 weeks (Zadoks et al., 2003). The present study′s findings are not dissimilar to those of studies in which persistent infections were evaluated in non-Jersey breeds.

Overall, there were 75 different microbes characterized to the genus and/or species level in this study, including many microbial genera and species that were infrequently isolated, with some being found only once in the study samples. Many of these low-frequency microorganisms have not been previously reported as known mastitis-associated pathogens. The IMI definition used in this study was selected in an effort to

TABLE 4. Least squares means from the linear mixed model showing the association of milk culture result and SCC[1] after accounting for DIM. The model included 6,211 observations from 2,794 quarters of 737 cows collected from the 4 herds sampled 3 times in consecutive monthly visits and included quarter, cow, and farm level clustering. IMI status is reported in descending order of overall prevalence by the most frequently encountered species or grouping of similar microorganisms. The study enrolled 4 all Jersey herds within 250 miles of Columbia, Missouri

IMI status	PREDICTED SCC	95% CI
Staphylococcus chromogenes	121[d]	89–165
Staphylococcus simulans	212[e]	158–285
Staphylococcus aureus	651[a, d, e, f]	138–3,060
Streptococcus dysgalactiae	1,658[a]	935–2,940
Streptococcus uberis	216[d, e]	96–486
Staphylococcus haemolyticus	64[b, c, d, e]	10–428
Staphylococcus gallinarum	181[d, e]	96–344
Serratia marcescens	268[e]	235–304
Staphylococcus xylosus	184[d, e]	130–260
Corynebacterium spp.[2]	53[c, f]	44–64
Other SSLO [3]	177[d, e]	95–329
Candida spp.[4]	225[e]	177–287
Bacillus spp.[5]	22[b, c]	11–43
Coliforms[6]	243[a, b, c, d, e]	97–613
Other NASM[7]	212[a, b, c, d, e]	31–1,436
No Significant Growth	35[b]	25–48

Estimates with different superscripts were statistically significant after adjustment for multiple comparisons. All $P \leq 0.01$.

[1] The SCC natural logarithm was used and back-transformation performed to report SCC rounded to whole cell number x10^3/mL.

[2] *Corynebacterium* spp.*, *Corynebacterium amycolatum, Corynebacterium ulcerans, Corynebacterium xerosis.*

[3] Includes other streptococcal and streptococcal-like organisms (SSLO) - *Streptococcus* spp.*, *Streptococcus canis, Streptococcus gallolyticus, Streptococcus lutetiensis,* strep-like species (*Aerococcus* spp.*, *Aerococcus viridans, Enterococcus faecalis, Enterococcus faecium, Lactococcus* spp.*, *Lactococcus garvieae, Lactococcus lactis*), excluding *Strep.* listed in table above.

[4] *Candida* spp.*, *Candida kefyr, Candida krusei, Candida lusitaniae, Candida rugosa, Candida tropicalis.*

[5] *Bacillus* spp.*, *Bacillus licheniformis, Bacillus pumilus, Bacillus sonorensis.*

[6] Includes coliform and gram-negative bacteria (*Enterobacter xiangfangensis, Escherichia coli, Proteus hauseri, Proteus vulgaris, Pseudomonas aeruginosa, Serratia ureilytica*), excluding *Serratia marcescens.*

[7] Includes other non-*aureus* staphylococcal and mammaliicoccal (NASM) species - *Staphylococcus agnetis/hyicus, Staphylococcus auricularis, Staphylococcus devriesei, Staphylococcus epidermidis, Staphylococcus equorum, Staphylococcus nepalensis, Mammaliicoccus sciuri, Staphylococcus warneri*, not listed in table above.

* Genus-level identification only.

optimize sensitivity and specificity for the majority of potential subclinical mastitis pathogens. The use of >1 colony/inoculum threshold for identification of species other than *Staph. aureus* and *Strep. agalactiae* could have led to over-detection of contaminants not associated with an IMI. It is also possible that some of these less common microbial genera and species were grouped among other common mastitis pathogen groupings in prior studies (ex: SSLO or coliforms), especially those studies where microbial identification was based largely on phenotype rather than molecular methods. Work is ongoing to understand the importance of these "newly" identified species in the context of bovine mastitis, and whether these microorganisms are potential udder pathogens or contaminants (Kurban et al., 2022).

A prior study investigating IMI prevalence in primiparous cows indicated that Jerseys tended to be in a higher SCC range than other breeds (including Swedish Holsteins) following individual assessment of the first 2 milk recordings (Persson Waller et al., 2020). Previous works reported overall mean cow-level test day SCC for Jerseys to be 212,000 cells/mL versus Holsteins at 167,000 cells/mL (Sewalem et al., 2006), and another study reported 100,709 cell/mL versus 84,965 cells/mL, respectively, using composite milk sample records (Berry et al., 2007). However, this breed-related discrepancy in average SCC has not been consistently observed, as shown in a large-scale nationwide study that did not report statistically significantly different average SCC between the 2 breeds (Capper and Cady, 2012). One explanation for the described difference is that average SCC may be higher for Jerseys due to the lower volume of milk produced. A 4-year study evaluating total lactation performance of dairy cows reported that Jerseys produce 23.3% less milk than Holsteins (White et al., 2002). Notably, in the present study, the AMS herd had an average cow annual milk production of almost twice that of the other herds and had the lowest bulk tank SCC of all the herds, potentially reflecting lower SCC correlating with greater milk volume produced. Ultimately, the present study did not provide substantial insight into or evidence for the supposition that Jerseys may demonstrate higher SCC than Holsteins, but it should be acknowledged that this study investigated quarter-level data, rather than cow-level data, as referenced in the aforementioned studies. Composite level samples are collected through milk meters and are routinely used in DHI programs; however, an individual quarter level SCC elevation may go undetected in a composite sample due to dilution from health quarter milk (Ruegg and Reinemann, 2002).

The reported farm bulk tank SCC at sampling times (which correlated similarly to cow-level test day values when available) were noticeably higher than the predicted SCC values for a given microbial genus or species, which on average, was lower than expected for IMI-positive quarters. A potential reason for our observed low SCC could be that the detected microorganisms were present in milk samples but not causing an IMI and eliciting an inflammatory response (i.e., the cultured microbes were contaminants from the skin or streak canal, which would have lowered the average SCC within microbial grouping). Further, if the inoculum volume of milk deposited from the cotton-tipped

TABLE 5. Quarter-level outcomes of 231 IMI at first sampling reported by milk culture result regarding persistent infection. IMI status is reported in descending order of overall prevalence by the most frequently encountered species or grouping of similar microorganisms. Percentages shown are the percent of quarters within each diagnosis (persistent or non-persistent) represented by the specific organism or organism grouping. The study enrolled 4 all Jersey herds, including a total of 753 cows, within 250 miles of Columbia, Missouri. Quarter milk samples were collected for 3 consecutive months from all lactating cows

Diagnosis	Persistent IMI (%)	Non-Persistent IMI (%)
Staphylococcus chromogenes	78 (42)	7 (15)
Staphylococcus simulans	50 (27)	2 (4)
Staphylococcus aureus	22 (12)	4 (9)
Streptococcus dysgalactiae	4 (2)	1 (2)
Streptococcus uberis	6 (3)	1 (2)
Staphylococcus haemolyticus	5 (3)	0 (0)
Staphylococcus gallinarum	3 (2)	1 (2)
Serratia marcescens	2 (1)	4 (9)
Staphylococcus xylosus	2 (1)	0 (0)
Corynebacterium spp.[1]	4 (2)	7 (15)
Other SSLO[2]	4 (2)	7 (15)
Candida spp.[3]	2 (1)	4 (9)
Bacillus spp. [4]	0 (0)	4 (9)
Coliforms[5]	2 (1)	3 (7)
Other NASM[6]	1 (<1)	1 (2)
Total	185	46

[1]*Corynebacterium* spp.*, *Corynebacterium amycolatum and Corynebacterium ulcerans*..

[2]Includes other streptococcal and streptococcal-like organisms (SSLO) - *Streptococcus* spp.*, *Streptococcus canis,* and strep-like species (*Enterococcus faecalis, Lactococcus garvieae, Lactococcus lactis*), excluding *Strep.* listed in table above.

[3]*Candida* spp.*, *Candida kefyr, Candida krusei, Candida tropicalis*..

[4]*Bacillus* spp.*, *Bacillus licheniformis, Bacillus sonorensis*..

[5]Includes coliform and gram-negative bacteria (*Enterobacter xiangfangensis, Pseudomonas aeruginosa*, *Serratia ureilytica),* excluding *Serratia marcescens*..

[6]Includes other non-*aureus* staphylococcal and mammaliicoccal (NASM) species - *Staphylococcus devriesei*, *Mammaliicoccus sciuri,* excluding *Staph.* listed in table above.

* Genus-level identification only.

applicator was greater than the approximated quantity of 10 μL, the sensitivity of microbial detection would be increased (reducing false-negative results), and specificity decreased (increasing false-positive results). It is also possible that there was some error introduced through handling of the quarter-level milk samples sent out for SCC, such as delays in shipment or shipment temperature fluctuations. While all of these factors are possible, they are likely consistent within the study allowing for study comparisons; however, potentially impacting the broader applicability of the data to a larger cohort of Jersey herds.

With regard to study limitations, the high rate of contaminated samples reduced the total number of available samples for the study. Sample contamination was an issue due to cow cleanliness and sample collection methods used (free stalls head lock sampling) on the AMS herd. Furthermore, ideally, we would have evaluated more farms and farms with similar production systems to reduce farm differences as much as possible. The small number of herds included in this study with the large variation in herd type limits the generalizability of the data. Given that the scope of this research was not focused on assessment of subclinical mastitis risk factors, it was less vital to have uniform management systems, albeit still important to consider. The longitudinal study design with repeated sampling of individual animals allowed us to focus on the microbial populations and SCC associations at the quarter level. Future studies should evaluate herds over a wider geographic area to more effectively understand Jersey cattle udder health at the national level. The persistence analysis was limited by the timeframe of the sampling period, so it was unknown if a given quarter was infected before the first sampling date or conversely if a late-detected infection persisted after the last sampling date. Study investigation of persistent infections could be further expanded in the future to include more samplings and strain typing. Only 65% of the cows were available for all 3 samplings due to lactation herd addition and attrition, and this hindered performing as comprehensive persistence analysis as intended. Utilization of a longer sample collection period would yield a greater number of data points for statistical inclusion, thus reducing the degree of statistical error. Additionally, decreasing the sampling interval could diminish the likelihood of new infections arising that could be falsely associated with infection persistence. Related to

this, identifying microbial strain would be beneficial to avoid making false conclusions of IMI persistence as infections with different strains of the same species can occur, as reported for NASM (Fry et al., 2014, Bernier Gosselin et al., 2019). Several IMI were only identified at the genus level, which may have resulted in underrepresentation of microorganisms of a given species. The plate extraction MALDI-TOF method was used, which yields similar identification percentage to the tube extraction MALDI-TOF protocol, but the latter method or alternative gene-based identification systems (housekeeping gene sequencing methods) could have been performed for instances when species identification was unsuccessful (Barcelos et al., 2019).

CONCLUSIONS

Intramammary infection prevalence was assessed and detailed in 4 enrolled Jersey herds. Non-*aureus* staphylococci, particularly *Staph. chromogenes* and *Staph. simulans*, were the most commonly identified microorganisms. Pathogen prevalence varied among farms but *Staph. chromogenes, Staph. aureus*, and *Strep. dysgalactiae* were identified on all farms. *Staphylococcus chromogenes* was one of the most prevalent microorganisms isolated on all farms. Overall, no major differences were found in this study from other similar studies in other dairy breeds. The quarter SCC values obtained in the present study do not appear to be meaningfully different than historical SCC values reported for Holsteins and therefore do not support the notion that Jersey cows have a higher SCC than Holstein cows. At this time, evidence suggests that Jersey farmers can anticipate IMI prevalence, microorganism-specific associations with SCC, or IMI persistence to be similar to that described in other breeds.

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