INTRAMAMMARY INFECTIONS IN LACTATING JERSEY COWS: PREVALENCE OF PATHOGENS AND ASSOCIATION OF PATHOGEN TYPE WITH MILK SOMATIC CELL COUNT AND PERSISTENCE OF INFECTION

A Thesis Presented to

the Faculty of the Graduate School

at the University of Missouri – Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

SAMANTHA ROSE HAW

Dr. John R. Middleton and Dr. Pamela R. F. Adkins, Thesis Advisors

JULY 2022

The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled:

INTRAMAMMARY INFECTIONS IN LACTATING JERSEY COWS:

PREVALENCE OF PATHOGENS AND ASSOCIATION OF PATHOGEN TYPE

WITH MILK SOMATIC CELL COUNT AND PERSISTENCE OF INFECTION

presented by Samantha R. Haw,

a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Dr. John R. Middleton, Thesis Advisor

_____

Dr. Pamela R. F. Adkins, Thesis Co-Advisor

_____

Dr. Scott E. Poock

_____

Dr. Véronique Bernier Gosselin

DEDICATION

This thesis is dedicated to my career mentors who have inspired and encouraged me over many years, observing my personal and professional growth and career transitions, offering unconditional support and care.

I also dedicate this thesis to Al and Maybelline. Without you, my journey would not have been the same. Thank you for your presence, love, and patience.

ACKNOWLEDGEMENTS

My first, well-warranted acknowledgement goes to my primary thesis advisors, Dr. Middleton and Dr. Adkins. I have been very fortunate to have had the opportunity to grow as a veterinary researcher under your tutelage. With your guidance, I learned how to write grants (with a 100% acceptance rate!), communicate my research findings, comprehend the benefits of abstract presentations and scientific posters, and greatly augment my dexterity with Excel. The depth and breadth of your research experience has been instrumental and invaluable in the development and completion of my thesis project, and for this, I sincerely thank you.

I would also like to acknowledge my other committee members. Dr. Poock, your involvement in the original grant provided by yourself and Dr. Adkins from the American Jersey Cattle Club Research Foundation was key in the initiation of my work. Dr. Bernier Gosselin, particular gratitude and recognition goes to you for providing the statistical analysis and persevering through the many challenges posed by my data.

Special acknowledgement and thanks are required for the hours of assistance provided by Dr. Adkins, Dr. Rivero, Dr. Walker, Kendelle Puga, Allena Radford, Madyson Mars, Tiffany Hagemann, and Natalie Sexton for sample collection and processing. My appreciation for your help is tremendous.

Thank you to the members of the Food Animal section, especially Dr. Hinds, for your support and encouragement during my residency training as I endeavored to succeed at being a full-time clinician and full-time student.

And finally, heartfelt acknowledgement goes toward my family, friends, and other colleagues who have shared in my journey. I'm glad you came along for the ride.
Thank you.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

CBA	Columbia Blood Agar
DHIA	Dairy Herd Information Association
DIM	Days in Milk
IMI	Intramammary Infection
lnSCC	Natural Logarithm Somatic Cell Count
MALDI-TOF	Matrix-Assisted Laser Desorption/ Ionization-Time-of-Flight
MS	Mass Spectrometry
NAS	Non-*aureus* Staphylococci
SCC	Somatic Cell Count

INTRAMAMMARY INFECTIONS IN LACTATING JERSEY COWS: PREVALENCE OF PATHOGENS AND ASSOCIATION OF PATHOGEN TYPE WITH MILK SOMATIC CELL COUNT AND PERSISTENCE OF INFECTION

Samantha Rose Haw

Dr. John R. Middleton, Thesis Advisor

Dr. Pamela R. F. Adkins, Thesis Co-Advisor

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, evaluate pathogen associations with somatic cell count (SCC) and persistence of IMI, and determine if the first sample SCC in a sequence of 3 samples could be predictive of IMI persistence with specific pathogens.

This prospective, observational, longitudinal study included lactating cows (n = 753) enrolled from 4 Jersey dairy farms within a 250-mile radius of Columbia, Missouri. Mammary quarter foremilk samples were aseptically collected once monthly for 3 consecutive months. Microorganisms were identified using aerobic milk culture and matrix-assisted laser desorption/ ionization-time-of-flight mass spectrometry. A commercial laboratory measured SCC using flow cytometry. Milk culture results were classified as single microorganism growth, mixed infection, contaminated, or no significant growth. Intramammary infection was defined based on isolation of > 1 colony

for all microorganisms except *Staphylococcus aureus* and *Streptococcus agalactiae*, where IMI presence was defined by isolation of ≥ 1 colony, or for *Bacillus* spp. when ≥ 5 colonies were isolated using an ~10μL inoculum. Persistent infection was characterized by identification of a single species-level pathogen at first sampling and at least 1 other visit. Non-persistent infections were those caused by a single species-level pathogen that was not subsequently encountered at another visit.

Non-*aureus* staphylococci, particularly *Staphylococcus chromogenes* and *Staphylococcus simulans,* were the most commonly isolated microorganisms among the 7,370 quarter-level milk samples collected. Median prevalence (using all 3 samplings) of specific pathogens varied among farms; however, *S. chromogenes* was 1 of the top 2 isolates found at all farms. There were some nuances observed between herds, likely attributable to farm management differences, that appeared to correlate with preponderances of certain microorganisms, specifically *S. simulans* and *S. aureus*. A linear mixed model evaluated the association of IMI status and natural logarithm SCC. An interaction with days in milk was observed for some species (*S. aureus*, *Streptococcus uberis, Serratia marcescens*, and *Corynebacterium* spp.). The predicted SCC associated with *Staphylococcus haemolyticus*, *Bacillus* spp., and coliforms were not significantly different from quarters with no growth. The most common species that persisted were *S. chromogenes, S. simulans, S. aureus,* and *Strep. uberis*. Mixed logistic regression revealed an association of natural logarithm SCC with IMI persistence (P = 0.04); however after pairwise comparison, there were no detectable differences in odds of persistence among pathogens. First sample period SCC was associated with persistent infection, but pairwise comparisons failed to show an association at the pathogen level.

Evaluating associations among commonly identified pathogens and SCC helped define how prevalent pathogens may associate with milk quality in Jersey cows. Overall findings shared some similarities with results of previously published studies, but overall SCC tended to be lower than other reports evaluating the same microbes among quarters. Some microbes were not significantly different from one another and showed similar alterations in SCC to uninfected quarters. *Staphylococcus aureus* and *Strep. dysgalactiae* were the most inflammatory causes of IMI. Dairy husbandry practices and milking hygiene are still critical control points for minimizing IMI and promoting udder health.

CHAPTER 1: INTRODUCTION

INTRODUCTION

MASTITIS

Mastitis, inflammation of the mammary gland, is the most common disease impacting the health and productivity of adult dairy cows (Ruegg, 2017). Mastitis significantly impacts dairy productivity costing the industry billions of dollars annually (Wells and Ott, 1998). The approximate cost of clinical mastitis in United States (US) dairy cattle is over $400 per case (Rollin et al., 2015, Liang et al., 2017). Mastitis is primarily caused by an IMI with bacteria or other microbial pathogens. Mastitis can be clinical (with changes in characteristics of the milk, mammary gland, and in severe cases the cow) or subclinical (with no observed clinical manifestations). Although subclinical mastitis is not associated with overt clinical disease, it can result in diminished quantity of milk produced as well as reduced milk quality (Halasa et al., 2009, Pantoja et al., 2009).

SOMATIC CELL COUNT AND PRODUCTION IMPACTS

Subclinical mastitis is the most common type of mastitis on dairy farms and is generally diagnosed by measuring the milk SCC. During IMI, this number serves as a proxy for neutrophils, which are present in large numbers during the inflammatory response to infection. The SCC can be affected by factors including breed, stage of lactation, parity, and IMI (Paape et al., 2001, Gonçalves et al., 2018, Sumon et al., 2020). Somatic cell counts < 200,000 cells/ml are typically associated with healthy, uninfected mammary glands (International Dairy Federation, 2013, Middleton et al., 2017).

Increased SCC can shorten the shelf-life of milk and reduce yields of milk-derived products such as cheese (Halasa et al., 2007, Potter et al., 2018). Because of these negative impacts, creameries desire low SCC milk, and monetary incentives for low SCC milk can improve dairy farm income. Conversely, consistently producing high SCC milk can lead to loss of market. Perhaps more insidious is the loss of income due to decreased overall milk production by cows with chronic subclinical mastitis (Martins et al., 2020). While a large body of work has been published over the years on subclinical mastitis, the vast majority has focused on Holstein cattle.

THE JERSEY BREED

Jersey cattle currently make up the second most prevalent breed of dairy cow in the US, following Holsteins. Jerseys make an important contribution to the US dairy economy with approximately 1.3 million head constituting about 14% of the total US dairy cattle population. Jersey milk consists of greater levels of fat, protein, calcium, and magnesium when compared to that of Holsteins, 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. Over the past several decades, there has been a decline in per capita consumption of fluid milk and an increase in consumption of cheese, whose manufacture utilizes over 40% of the milk produced in the US (Capper and Cady, 2012). As consumer trends have resulted in an increasing demand for cheese, the distribution of breeds in the US dairy cattle population has simultaneously shown an increase in the number of Jersey cattle (Council on Dairy Cattle Breeding, 2018).

Jersey numbers may continue to increase as global markets focus more on effective and sustainable food production. Jerseys offer the benefits of greater feed efficiency, lower consumption of natural resources, and a reduced carbon footprint (Capper and Cady, 2012, Gonzalez-Peña et al., 2020). Given the increasing number of Jersey cattle in the US and the breed-specific advantages they offer in dairy farming and food production, it is important to expand the existing body of knowledge to further address the impact of subclinical mastitis on udder health and dairy production in Jersey cows.

FOCUS OF SUBCLINICAL MASTITIS RESEARCH

The majority of subclinical mastitis research has been conducted in Holstein cattle or other non-Jersey breeds (Halasa et al., 2009, Bobbo et al., 2017). 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 both acute and chronic subclinical mastitis in Jerseys as well as how specific microbial pathogens potentially influence SCC measurements.

INTRAMAMMARY INFECTION

The udder immune response and extent to which SCC increases has been shown to differ based on the pathogen causing the IMI. Intramammary infection, presence of an infectious organism in milk, is primarily caused by bacteria. *Staphylococcus* is the most common bacterial genus isolated from cow milk and typically causes subclinical mastitis. *Staphylococcus aureus* usually causes moderate to severe increases in SCC and is categorized as a major mastitis pathogen. The NAS group, on the other hand, historically

had been thought to only cause a slight SCC elevation in affected quarters relative to uninfected quarters, and the NAS are categorized as minor mastitis pathogens (Middleton et al., 2017).

NAS have become the most commonly isolated bacteria from cases of subclinical mastitis in well-managed dairy herds (Pitkälä et al., 2004, Tenhagen et al., 2006, Condas et al., 2017a). Within the NAS group, there are differences among species with regard to their impact on udder health (Vanderhaeghen et al., 2015). In Holstein cattle, *Staphylococcus chromogenes* and *Staphylococcus simulans* are 2 of the most prevalent species associated with IMI and have been associated with persistent infections and significantly higher SCC (Fry et al., 2014). Despite the characterization as a minor mastitis pathogen, there is increasing evidence that some of these species may cause SCC elevations comparable to those of major pathogens (Supré et al., 2011, Valckenier et al., 2020).

Similar pathogen-specific differences in SCC alterations are also seen in cases of subclinical mastitis caused by other bacteria such as *Corynebacterium* spp., a minor mastitis pathogen. Corynebacterium-infected mammary quarters had higher SCC than healthy quarters, but elevations were only associated with *Corynebacterium bovis,* whereas there was no change in SCC with non-*C. bovis* quarters (Gonçalves et al., 2016).

SUBCLINICAL MASTITIS PATHOGEN PREVALENCE

Currently, generalized prevalence data of subclinical mastitis pathogens in US dairy cattle is limited. Existing prevalence studies often focus on a particular bacterial genus (Tomazi et al., 2015, Gonçalves et al., 2016, Sun et al., 2017). In Canada, a few

large studies have been published on the distribution of NAS IMI and its association with subclinical mastitis (Fry et al., 2014, Condas et al., 2017b). However, these Canadian studies investigating NAS and inflammation have primarily involved Holstein cattle (Fry et al., 2014, Condas et al., 2017b), as did another smaller study conducted in Portugal (Bexiga et al., 2014). Most of the existing studies that explore subclinical mastitis pathogen-specific effects (Valckenier et al., 2019, Martins et al., 2020) have failed to explain breed-associated differences in SCC. One such example is the reportedly higher average SCC in Jersey cows than in Holstein cows based upon test-day records (Sewalem et al., 2006, Berry et al., 2007). It is unclear if these breed differences in SCC are attributable to variations in the immune response to IMI with different pathogens or are associated with the lower amount of milk produced by Jersey cattle relative to Holsteins.

KNOWLEDGE GAPS AND OBJECTIVES

Expanding the body of Jersey-specific data evaluating subclinical mastitis will fill an important gap in our understanding of factors that potentially influence milk quality in Jerseys. By understanding the potential drivers for elevated milk SCC in Jersey cattle with subclinical mastitis, dairy producers and veterinarians can work to develop targeted mastitis control strategies to reduce the prevalent microbial populations and minimize their impact on cow-level and herd-level (bulk tank milk) SCC. These efforts will in turn be translated into more milk produced, longer shelf life, and improved dairy product yields.

Jersey milk generates greater cheese product yields and increased manufacturing plant efficiency, both of which may contribute to further increased demand for Jersey cows (Stocco et al., 2018). Jersey cattle have a more compact body frame, offer greater

feed efficiency for milk production, use fewer agricultural resources and generate less waste (Gonzalez-Peña et al., 2020). Compared to Ayrshire, Brown Swiss, Guernsey, and Holstein cows, Jerseys have the longest productive herd life and survivability with greatest mean number of calvings (Hare et al., 2006). With an increased worldwide demand for wholesome animal-source foods, the Jersey breed offers some distinct advantages over the Holstein breed and understanding breed-specific associations between IMI and milk SCC are important to ensuring profitability and cow welfare.

MAIN OBJECTIVES

- Characterize the prevalence of IMI caused by different microorganisms in lactating Jersey cattle.
- Evaluate pathogen-specific associations with SCC and persistence of IMI.
- Determine if first SCC sample in a sequence can be predictive of pathogen-specific IMI persistence.

CHAPTER 2: 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 recorded from DHIA data, unless these data were absent from individual cow records. The study protocol (#9896) was approved by the University of Missouri Animal Care and Use Committee.

MILK SAMPLE COLLECTION

Mammary quarter foremilk samples were collected from all functional quarters of all lactating cows during routine milking approximately once monthly for 3 consecutive months. Herd enrollment dates ranged from September 2020 through January 2021. Prior to sample collection, farm personnel disinfected and dried teats according to each farm's established pre-milking hygiene protocol, with exception of cows from 1 herd with an automated milking system, where cows were sampled between milkings. Sample collection was performed while wearing disposable nitrile gloves. The apex and barrel of each teat were scrubbed with a 70% isopropyl alcohol-soaked non-woven cotton gauze. Each quarter was manually stripped of a few streams of milk before aseptically collecting milk into a sterile plastic vial for bacterial culture (Middleton et al., 2017, Wattenburger et al., 2020). Immediately after, milk from each mammary quarter was collected into separate nonsterile vials containing a 2-bromo-2-nitropropane-1, 3-diol preservative tablets (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 bacterial cultures were frozen (-20°C) within 8 hours of collection until cultured. 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

Milk samples were stored for 1-25 days prior to culture. Samples were thawed at room temperature (~22°C) prior to 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 (CBA) 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 hours. 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, and isolates were not further evaluated.

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 direct transfer method (Cameron et al., 2017) 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, 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 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. 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.

SCC ENUMERATION

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

DEFINITIONS

Definitions of IMI were based on an assumption of inoculum size of ~10 µL and adapted from Dohoo et al., 2011. A mammary quarter was defined as having an IMI if > 1 colony (Dohoo et al., 2011) was isolated on CBA 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 CBA after 48 hours or if colony counts were below the aforementioned 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 phenotypically assessed to be an IMI that did not yield a genus or species were categorized as unidentified in the descriptive analysis. Missing samples, blind mammary quarters, unidentified microorganisms, mixed infections, and contaminated samples were considered as missing (censored) data in the evaluation of persistence of IMI and the statistical analyses.

For the evaluation of persistence of IMI in a given mammary quarter, an IMI was considered persistent when the same pathogen, identified to the species level, was isolated at first sampling and at least 1 other sampling. Intramammary infections were considered not persistent if first sampling yielded a single species-level pathogen that was not subsequently encountered at another visit.

DATA ANALYSIS

Using the definitions above, descriptive data were assembled into a table displaying overall occurrence of each outcome across all samplings within herd. In

addition, median prevalence of each outcome was calculated using data from all 3 visits to each farm. Persistent infections were tabulated for descriptive purposes.

Statistical models (described below) were constructed to evaluate the association between pathogen-specific IMI and milk SCC and to evaluate the ability of first sample milk SCC to predict persistence of IMI with a given pathogen. To improve model convergence, the list of quarter-level outcomes was narrowed to known mastitis pathogens that occurred more than 10 times in the overall data set. Categories included microbial species-level data for the most frequently encountered organisms. *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). No significant growth was included as a negative comparator group. The SCC natural logarithm (lnSCC) was used for statistical analysis.

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, cow, and farm. Data was 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, $\geq$ 5), and days in milk (including polynomial terms) were evaluated for inclusion in the model. Associations between independent variables were assessed, as were interactions in reduced models. A significant difference was defined as $P < 0.05$. Statistically significant

interactions were evaluated in the model. Normality and homoscedasticity of residuals were visually assessed. Sidak adjustment was used for multiple comparisons.

Using data from infected quarters on the first sampling, a mixed logistic regression model was built to evaluate the association between persistence status (dependent variable) and pathogen-specific (species-level) IMI status and lnSCC at the first sampling visit, 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. Quarters in which the first and third sampling outcomes yielded the same pathogen but no IMI at the second sampling were evaluated as if the same pathogen were present throughout. Unconditional relationships between persistence and lnSCC, IMI species group, parity (as above), and days in milk were evaluated. Associations between independent variables were assessed, as were interactions in reduced models. Statistically significant interactions were evaluated in the model, which included *S. simulans, S. aureus, Corynebacterium* spp., and the group of *Streptococcus* spp. and *Strep*. -like spp. Sidak adjustment was used for multiple comparisons. Quarters not sampled at first farm visit and quarters only sampled at first visit were excluded from analysis. Quarters yielding a censored result (unidentified microorganism, mixed infection, and contaminated sample) at first visit were also excluded.

Analyses were conducted using STATA version 16.1 (StataCorp LLC, College Station, TX).

CHAPTER 3: RESULTS

Herd sizes ranged from 92-280 lactating cows. Table 3.1 describes herd characteristics including herd size, housing style, milking facilities, average annual production per cow, number of animals and quarters sampled per visit, and average bulk tank SCC at each sampling period. Enrolled herds included a mixture of confinement and outdoor housing. Three herds had milking parlors and 1 utilized an automated milking system.

A total of 753 cows (2,996 mammary quarters) were enrolled and 7,370 quarter-level milk samples were collected over the 3 sampling periods. Sixteen mammary 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 at 2 samplings, and 111 (15%) cows had samples collected once.

Overall mammary quarter-level milk culture results for each herd are reported in Appendix 1.

Staphylococcus chromogenes, S. aureus, and *Streptococcus dysgalactiae* were identified on all 4 farms. No Significant Growth was the most frequent outcome (5,543/7,370; 75.2%). Non-*aureus* staphylococci were among the most frequently identified organisms. *Staphylococcus chromogenes* was the most common microorganism (320/7,370) isolated overall but not always the most common found within herd, being the most common in herds 1 and 2, whereas *S. aureus* prevailed in herd 3 and *S. simulans* prevailed in herd 4. *Corynebacterium amycolatum* was the fourth-most common microorganism (36/7,370) identified at the species level. *Streptococcus*

agalactiae was isolated once. A large number of organisms (31) were infrequently isolated (fewer than 15 times overall; Appendix 2).

TABLE 3.1. Herd management and production information in addition to number of cows (and quarters) sampled and bulk tank SCC for each herd visit.

	HOUSING STYLE	MILKING FACILITIES	AVERAGE ANNUAL MILK PRODUCTION (kg)	COWS[1] (QUARTERS) SAMPLED AND BULK TANK SCC (cells/mL)		
				VISIT 1	VISIT 2	VISIT 3
Herd 1	Confinement with sand-bedded free stall	Parlor, double 10 herringbone	7,394	246 (969)	250 (973)	240 (933)
				227,500	232,000	148,000
Herd 2	Dry lot and pasture	Parlor, double 5 herringbone	6,932	110 (430)	105 (411)	105 (413)
				388,000	233,000	315,000
Herd 3	Pasture and loose housing	Parlor, double 4 herringbone	6,030	80 (312)	75 (293)	77 (281)
				340,000	385,000	680,000
Herd 4	Confinement with sand-bedded free stall	Automatic milking system (robotic)	12,161	207 (808)	196 (756)	200 (786)
				150,000	160,000	140,000

[1] Number represents lactating population.

The median prevalences of quarter level outcomes calculated using all 3 samplings for each herd are reported in Table 3.2.

TABLE 3.2. Median (range) quarter-level prevalence as a percentage calculated using all 3 samplings within each herd organized by isolated microbial groups in descending order of prevalence.

Diagnosis	**Herd 1 %**	**Herd 2 %**	**Herd 3 %**	**Herd 4 %**
No Significant Growth	86.3 (83-88.5)	76.7 (68.8-80.8)	70.3 (64.7-72)	64.9 (59.3-68.7)
Staphylococcus chromogenes	2.5 (2.4-2.6)	5.6 (4.6-5.6)	6.1 (5.4-7)	5 (4.8-6.6)
Staphylococcus simulans	0 (0-0)	0 (0-0.2)	0 (0-0)	8.7 (8-8.9)
Staphylococcus aureus	0.2 (0.1-0.3)	0.7 (0.5-2.3)	6.8 (6.7-8)	0.1 (0-0.1)
Streptococcus dysgalactiae	0.7 (0.-0.7)	0 (0-0.5)	0.7 (0.3-1.7)	0.2 (0.1-0.8)
Streptococcus uberis	0.5 (0.3-0.6)	0.5 (0.5-0.7)	0 (0-0.3)	0 (0-0)
Staphylococcus haemolyticus	0 (0-0)	0.9 (0.5-1)	0 (0-0)	0.4 (0.3-0.5)
Staphylococcus gallinarum	0.2 (0.1-0.4)	0.2 (0.2-0.5)	0 (0-0)	0.2 (0.1-0.4)
Serratia marcescens	0.1 (0.1-0.2)	0.5 (0.2-1.9)	0 (0-0.3)	0 (0-0)
Staphylococcus xylosus	0 (0-0)	0.2 (0.2-0.2)	0 (0-0)	0.4 (0.4-0.4)
Corynebacterium spp.[1]	0.1 (0.1-0.6)	1.4 (1-1.7)	0.3 (0-0.7)	0.9 (0.7-2.2)
Streptococcus and *Strep*. -like spp.[2]	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]	0.2 (0.2-0.8)	0.5 (0.2-1.2)	0 (0-0.3)	0 (0-0)
Bacillus spp.[4]	0.4 (0-0.4)	0 (0-0.2)	0 (0-0.3)	0 (0-0)
Coliforms[5]	0.1 (0.1-0.3)	0.5 (0.2-1)	0 (0-0.3)	0.4 (0.3-0.6)
Other NAS[6]	0.1 (0-0.3)	0.2 (0-0.5)	0.3 (0-0.3)	0.4 (0.1-0.6)
Undetermined Identity	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	0.5 (0.4-0.6)	1.2 (0.5-1.6)	1.4 (0-2)	0.8 (0.5-1.4)
Contaminated	6.5 (3.4-7.1)	4.1 (4-17.7)	7.8 (5.9-18.3)	17.2 (8.9-22.2)

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

[2] Includes *Streptococcus* spp.*, *S. canis, S. gallolyticus, S. lutetiensis,* Strep-like species (*Aerococcus* spp.*, *A. viridans, Enterococcus faecalis, E. faecium, Lactococcus spp.*, L. garvieae, L. lactis*), excluding *Strep.* listed in table above.

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

[4] Includes *Bacillus* spp.*, *B. licheniformis, B. pumilus, B. 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 NAS (*S. agnetis/hyicus, S. auricularis, S. devriesei, S. epidermidis, S. equorum, S. nepalensis, S. sciuri, S. warneri*), not listed in table above.

* Genus-level identification only

The prevalence of each outcome varied slightly among farms, but *S. chromogenes* was among the top 2 most commonly encountered organisms found at all farms. Overall, 14 different *Staphylococcus* spp. were identified. The relative frequency of diagnosis of each *Staphylococcus* spp. is shown in Table 3.3.

The grouped *Corynebacterium* spp. were in the top 3 IMI for farms 2 and 4. No *C. bovis* was detected. Other *Streptococcus* spp. and *Strep.* -like species were in the top 3 IMI for farms 1 and 3. *Streptococcus dysgalactiae* was encountered in the top 4 organisms in herds 1 and 3.

TABLE 3.3. Frequency of diagnosis of *Staphylococcus* spp. IMI outcomes among all 3 samplings within herds with percent of each *Staphylococcus* organism per herd shown parenthetically.

Diagnosis	**Herd 1**	**Herd 2**	**Herd 3**	**Herd 4**
Staphylococcus chromogenes	72 (79.1)	66 (64.1)	57 (46.3)	129 (34.8)
Staphylococcus simulans	0 (0.0)	1 (1.0)	0 (0.0)	205 (55.3)
Staphylococcus aureus	7 (7.7)	15 (14.6)	64 (52.0)	2 (0.5)
Staphylococcus haemolyticus	0 (0.0)	10 (9.7)	0 (0.0)	10 (2.7)
Staphylococcus gallinarum	7 (7.7)	4 (3.9)	0 (0.0)	7 (1.9)
Staphylococcus xylosus	0 (0.0)	4 (3.9)	0 (0.0)	9 (2.4)
Staphylococcus 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	**91**	**103**	**123**	**371**

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

Observations were omitted from statistical analyses due to absence of the following information: cow ID (4), parity (7), DIM (86), SCC (148). There were 1106 observations omitted due to identification as: unidentified microorganism (84), mixed

infection (60), no sample collected (190), contaminated sample (742); the remaining excluded data points were due to microorganisms being infrequently encountered (30). A single occurrence of *Streptococcus agalactiae* was excluded due to inability to adequately perform statistical analysis. After exclusion of the aforementioned data, 6,211 observations from 2,794 mammary quarters of 737 cows were available for inclusion in the linear mixed model. Parity and polynomial terms of DIM were significant in unconditional analyses, but no longer significant in the final model. An interaction ($P < 0.01$) of SCC with DIM was observed for some IMI categories, including *S. aureus*, *Streptococcus uberis*, *Serratia marcescens*, *Corynebacterium* spp., and *Candida* spp. The association of pathogen-specific IMI with SCC is shown in Table 3.4. Many IMI pathogen species/ groups were associated with a predicted SCC less than 200,000 cells/mL. The highest predicted SCC were associated with IMI caused by *S. aureus* and *Strep. dysgalactiae*. The predicted SCC associated with *S. haemolyticus*, *Bacillus* spp., and coliforms were not significantly different from quarters with no growth.

Data considered for inclusion in the mixed logistic regression model for evaluation of IMI persistence are described in Table 3.5. There were 185 IMI that met the criteria for persistent. The most common bacterial species associated with persistent IMI were *S. chromogenes*, *S. simulans*, *S. aureus*, and *Strep. uberis*.

There were 39 IMI that were classified as non-persistent infections. Excluded from the analysis were 10 quarters with distinct IMI pathogens at each visit and 9 quarters with 2 IMI of different pathogens and a censored IMI result across samplings.

TABLE 3.4. Least square means from the linear mixed model showing the association of culture result and SCC[1] after accounting for DIM. The model included 6,211 observations from 2,794 quarters of 737 cows and included quarter, cow, and farm level clustering.

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
Streptococcus and *Strep*. -like spp.[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 NAS[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] Includes *Corynebacterium* spp.*, *C. amycolatum, C. ulcerans, C. xerosis.*

[3] Includes *Streptococcus* spp.*, *S. canis, S. gallolyticus, S. lutetiensis*, Strep-like species (*Aerococcus* spp.*, *A. viridans, Enterococcus faecalis, E. faecium, Lactococcus spp.*, L. garvieae, L. lactis*), excluding *Strep.* listed in table above.

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

[5] Includes *Bacillus* spp.*, *B. licheniformis, B. pumilus, B. 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 NAS (*S. agnetis/hyicus, S. auricularis, S. devriesei, S. epidermidis, S. equorum, S. nepalensis, S. sciuri, S. warneri*), not listed in table above.

* Genus-level identification only

TABLE 3.5. Quarter level outcomes reported by culture result regarding persistent infection and inclusion in mixed logistic regression model.

Diagnosis	Persistent IMI	Non-Persistent IMI	Censored quarter	Quarters included in analysis
Staphylococcus chromogenes	78	6	16	84
Staphylococcus simulans	50	2	4	52
Staphylococcus aureus	22	4	3	26
Streptococcus dysgalactiae	4	0	5	4
Streptococcus uberis	6	0	0	6
Staphylococcus haemolyticus	5	0	3	5
Staphylococcus gallinarum	3	1	0	4
Serratia marcescens	2	3	3	5
Staphylococcus xylosus	2	0	2	2
Corynebacterium spp.[1]	4	5	10	9
Streptococcus and *Strep*. -like spp.[2]	4	7	6	11
Candida spp.[3]	2	3	1	5
Bacillus spp.[4]	0	4	1	4
Coliforms[5]	2	3	0	5
Other NAS[6]	1	1	0	2
Total	**185**	**39**	**54**	**224**

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

[2] Includes *Streptococcus* spp.*, *S. canis, S. gallolyticus, S. lutetiensis,* Strep-like species (*Aerococcus* spp.*, *A. viridans, Enterococcus faecalis, E. faecium, Lactococcus spp.*, L. garvieae, L. lactis*), excluding *S. dysgalactiae and S. uberis,* excluding *Strep.* listed in table above.

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

[4] Includes *Bacillus* spp.*, *B. licheniformis, B. pumilus, B. sonorensis.*

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

[6] Includes Other Non- *aureus* Staphylococcus (*S. agnetis/hyicus, S. auricularis, S. devriesei, S. epidermidis, S. equorum, S. nepalensis, S. sciuri, S. warneri*), not listed in table above.

* Genus-level identification only

There were 976 mammary quarters with no growth at all 3 samplings. Of these, 284 quarters were from 71 cows with no growth in any teat at all 3 samplings.

The mixed logistic regression model to evaluate if first sampling lnSCC could be used to predict persistence of infection included data from 224 quarters on 162 cows. There were 21 quarters in which the first and third sampling outcomes yielded the same pathogen but were culture negative at the second sampling. One quarter had the same pathogen at the first and third sampling but a different organism at second sampling and was evaluated as if a mixed infection (censored data point) were present at the middle sampling. Parity and DIM were not significant in unconditional analyses.

In the final model, the association between persistent infection and lnSCC was statistically significant (P = 0.04). Furthermore, the odds of persistence of IMI caused by *Corynebacterium* spp. or by the group of *Streptococcus* spp. and *Streptococcus*-like species were lower compared to those of *S. chromogenes* IMI ($P \leq 0.01$). After adjusting for multiple comparisons between species groups, there were no significant differences. However, IMI caused by the group of *Streptococcus* spp. and *Streptococcus*-like species tended to have lower odds of persistence than *S. simulans* ($P = 0.07$) and *S. chromogenes* IMI ($P = 0.09$), as did *Corynebacterium* spp. IMI compared to *S. simulans* IMI ($P = 0.08$).

CHAPTER 4: DISCUSSION

DISCUSSION

To the authors' knowledge, there have been no similar characterizations of subclinical mastitis in Jersey cows. The present investigation provides combined information about the prevalence of IMI caused by different microorganisms in lactating Jersey cattle and the association between individual pathogen species, SCC, and persistence of IMI.

Non-*aureus* staphylococci were among the most frequently encountered organisms identified. These findings are similar to previous reports in other dairy cattle breeds (Heikkilä et al., 2018, Zigo et al., 2022). NAS have become the most commonly isolated organisms from cow milk samples (Adkins et al., 2018). Some explanations for increased occurrence of NAS on farms have cited decreasing incidence of mastitis caused by major pathogens and increasing resistance among NAS to antimicrobials and disinfectants (Condas et al., 2017b, Zigo et al., 2022).

Staphylococcus chromogenes and *S. simulans* were among the most commonly isolated microorganisms in this study. *Staphylococcus chromogenes* was found to be 1 of the top 2 pathogens at all farms. *Staphylococcus chromogenes* was also the predominant species found among NAS in other studies (Piessens et al., 2011, Mørk et al., 2012, Tomazi et al., 2015). This pathogen has been associated with higher SCC when compared with culture negative quarters (Adkins et al., 2018, De Buck et al., 2021), which was similar to our findings in this study. Other studies, however, have found notably higher SCC (above 300,000 cells/mL) in *S. chromogenes* infected quarters (Tomazi et al., 2015,

De Visscher et al., 2016). *Staphylococcus simulans* has previously been found to be associated with SCC over 200,000 cells/mL (Supré et al., 2011, Fry et al., 2014), which was a shared outcome with the present study. The predicted SCC of quarters positive for *S. simulans* was statistically different and greater than SCC of *S. chromogenes* infected quarters, similar to other reports (Condas et al., 2017b). Although the focus of this study was not risk factor assessment, it was evident there were some differences in outcomes among farms. All occurrences of *S. simulans*, except 1, were isolated from the farm site that utilized an automated milking system. Specific risk factors for *S. simulans* IMI in automated milking system herds has not been reported.

In addition to *S. chromogenes* and *S. simulans*, the NAS commonly associated with IMI in other studies include *S. haemolyticus, S. epidermidis* (Mahmmod et al., 2018) and *S. xylosus* (Thorberg et al., 2009, Vanderhaeghen et al., 2014). The results of the present study included *S. haemolyticus, S. gallinarum*, and *S. xylosus* among the most commonly identified species. Condas et al. (2017b) reported that samples positive for most NAS species typically had lower SCC than quarters infected with the major pathogens of *S. aureus, Strep. dysgalactiae*, and *Strep. uberis.* In the present study, *S. chromogenes, S. simulans, S. haemolyticus, S. gallinarum*, and *S. xylosus* were different from *Strep. dysgalactiae* but not *S. aureus* or *Strep. uberis*. Following pair-wise comparison, NAS IMI caused by the grouping containing *S. agnetis/hyicus, S. auricularis, S. devriesei, S. epidermidis, S. equorum, S. nepalensis, S. sciuri*, and *S. warneri*, showed no difference when compared to *S. aureus, Strep. dysgalactiae*, or *S. uberis*. This finding was surprising, given that *S. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* often induce significant subclinical or clinical mastitis and substantial udder

inflammation (Reyher et al., 2012), so one would have expected to encounter a significant difference in pathogen-specific response. It is possible that in some of these quarters, the microbe was present as a contaminant in the milk sample but not actually causing disease or invoking an inflammatory response. The SCC of *S. haemolyticus* and the other NAS species group were not, on average, different from uninfected control mammary quarters, as reported by others (Fry et al., 2014). *Staphylococcus chromogenes, S. simulans, S. gallinarum* and *S. xylosus*, however, showed SCC associations with IMI that differed from uninfected quarters. Somatic cell alterations of *S. gallinarum* and *S. xylosus* were not different from that of *S. chromogenes* or *S. simulans*. This has been seen previously for *S. xylosus* (Supré et al., 2011). Supré et al. (2011) reported that *S. xylosus* infected quarters increased the SCC similar to what was seen in *S. aureus* infected quarters, which was supported in the present study. Our results coincide with past observations that NAS IMI have varying association with SCC that can range from minimal to more robust inflammatory responses but ultimately, *S. chromogenes* has been found to be the most common NAS pathogen associated with an elevated SCC.

Staphylococcus aureus was the third most detected pathogen. One farm had the majority of the *S. aureus* isolates. At that site, *S. aureus* presence was likely attributable to observed suboptimal milking hygiene practices such as lack of glove use, repeated utilization of towels for cleaning teats of multiple cows, and submerging the milking claw in a common bucket full of diluted bleach. This farm also recorded higher bulk tank SCC than the other farms, most likely attributable to *S. aureus* IMI. Identifying the presence of this contagious organism can be crucial for reducing production losses, as it can lead to long-standing infections that are refractory to treatment and causes elevations in SCC.

Quarters infected with *S. aureus* can have elevated SCC prior to onset of actual clinical mastitis and continue to have high SCC into the future (de Haas et al., 2002). Coincidentally, some of the quarters that were sampled in our study appeared to cure *S. aureus* infections yet continued to have elevated SCC in the later sampling periods at which time no microorganism growth was detected. In the present study, quarters infected with *S. aureus* demonstrated notable SCC elevations, however statistically these IMI were not different than IMI of other infected quarters.

Corynebacterium amycolatum was the fourth most frequently encountered organism, thus the *Corynebacterium* genus was the second most common overall, after staphylococci. There were no isolates of *C. bovis,* but other *Corynebacterium* spp. including *C. ulcerans* and *C. xerosis* were found. These organisms were more commonly encountered than what has been reported in other studies, where *C. bovis* predominated relative to other *Corynebacterium* spp. (Gonçalves et al., 2016, Lücken et al., 2021). One large survey study of udder health in Germany encountered *Corynebacterium* spp. as the second most common pathogen group following NAS (Schwarz et al., 2010), which is similar to this study. Minor pathogens, like *Corynebacterium* spp., are typically found to be associated with much less inflammation in comparison to major pathogens (Reyher et al., 2012). Findings from this study indicated that IMI of the *Corynebacterium* spp. group were different from uninfected quarters, with much lower predicted SCC, but SCC was similar to that reported previously for *Corynebacterium* spp. other than *C. bovis* (Lücken et al., 2021). *Corynebacterium* spp. do not usually cause substantial reductions in milking volume (Reyher et al., 2012, Gonçalves et al., 2016). That being said, it is notable that some research has shown that minor pathogens can still cause a negative effect on milk

production and should not be underestimated in their potential economic impact (Heikkilä et al., 2018).

Environmental streptococci, *Strep. dysgalactiae* and *Strep. uberis*, followed *C. amycolatum* in frequency of occurrence. These organisms are primarily environmental pathogens (Alnakip et al., 2020). Cattle affected with clinical mastitis due to environmental streptococci suffer substantial losses in milk yield in comparison to healthy animals or those infected with minor mastitis pathogens (Gonçalves et al., 2020). *Streptococcus uberis* is commonly associated with subclinical IMI (Zadoks et al., 2003, Keane, 2019). It is important for producers to be aware of the presence of these IMI pathogens and target environmental risk factors to mitigate infection by implementing appropriate milking and environmental hygiene practices. Quarters infected with *S. aureus* and *Strep. dysgalactiae* were found to be highly inflammatory in the present study and yielded significant predicted SCC alterations, as expected for these major mastitis pathogens. They were not different from one another when assessed with pairwise comparison, a finding that coincides with prior research (de Haas et al., 2002). *Streptococcus uberis* IMI were different from these other major pathogens but mean predicted SCC was only slightly over 200,000 cells/mL in the present study. Intramammary infections with other *Strep.* and *Strep.* -like spp. showed SCC associations that differed from control quarters but did not differ from *S. chromogenes* or *S. simulans.*

Serratia marcescens, a coliform bacterium, was also encountered somewhat frequently among infected quarters (16 times overall). This species has been reported to be the most common *Serratia* spp. isolated from bovine milk (Todhunter et al., 1991). *Serratia* spp. IMI can result in clinical or subclinical mastitis, but occurrence is typically

rare (Schukken et al., 2012). The presence of this pathogen can be correlated with environmental contamination and milking hygiene. Outbreaks of *S. marcescens* have been associated with contaminated teat-dip containers and milking equipment (Schukken et al., 2012, Friman et al., 2019). Quarters with *Serratia marcescens* IMI in the present study were associated with a higher SCC than uninfected quarters, similar to cow-level findings reported from a multistate outbreak (Schukken et al., 2012). In this study, *Serratia marcescens* IMI were not significantly different from IMI infected with other coliforms; however, following pair-wise comparison, the coliform IMI group was not found to be associated with significant changes in SCC relative to quarters with no growth. Quarters infected with coliforms of the *Enterobacter* genus were associated with the highest mean SCC relative to all other pathogens and uninfected quarters in a recent report (Sumon et al., 2020). Overall, the SCC findings in our study were surprising in comparison to what was expected of coliform pathogens. This may be explained by low pathogen load, early detection of infection prior to incitement of significant inflammatory response, or contamination.

Sumon et al. (2020) also reported *Bacillus* spp. to be the IMI group with the second-highest SCC, which was very different from the results we encountered. Here, the *Bacillus* group yielded the lowest mean predicted SCC and was not different from uninfected mammary quarters. It is possible these bacteria were detected as incidental contaminants rather than primary invading organisms causing IMI. *Bacillus cereus* has been associated with acute, gangrenous mastitis (Schiefer et al., 1976, Jones and Turnbull, 1981); however, none of these organisms were detected in our study.

Candida yeast IMI were associated with alteration in predicted SCC slightly above 200,000 cells/mL. Typically, these organisms are less common causes of mastitis but seem to be detected with increasing frequency in several countries (Dworecka-Kaszak et al., 2012, Milanov et al., 2014, Du et al., 2018). *Candida albicans* is one of the more common causes of fungal mastitis but other species can also result in disease. One study in China assessed milk samples from cattle with clinical mastitis and almost a quarter of samples were infected with other non-*albicans Candida* species (Du et al., 2018). In our present study, *Candida* spp. were isolated with low prevalence but also consisted of non-*albicans* species, including *C. kefyr*, *C. krusei, C. lusitaniae, C. rugosa*, and *C. tropicalis*. The *Candida* spp. isolates in the present study were primarily found at 2 herd sites; the reasoning for this distribution is unclear. There are no specific reported risk factors for *Candida*-associated IMI presence in these herds; however it may reflect farm site differences that led to more opportunistic fungal infection.

There were 31 different microorganism genus and species that were infrequently isolated. Some of these microorganisms were found only once in the study sampling. Many of these low-frequency microorganisms have not been previously reported as known mastitis-associated pathogens. The modified IMI definition used in this study was constructed in an effort to target relevant growth, necessitating > 1 colony/inoculum for species other than *S. aureus* and *Strep. agalactiae.* Hence, if our inoculum was >10µL, some of the unusual species may have been diagnosed due to detecting contaminants in low numbers or because these microorganisms would have historically not been named because speciation methods other than MALDI-TOF were used that were less able to

differentiate some of these unusual microbial species from other more common groupings of potential pathogens.

The most common persistent species were *S. chromogenes, S. simulans, S. aureus*, and *Strep. uberis*. Past works have noted *S. chromogenes* to be more commonly associated with persistent IMI in comparison to other NAS species (Valckenier et al., 2020). In addition to *S. chromogenes, S. simulans* has also been reported to cause persistent IMI (Taponen et al., 2007, Fry et al., 2014). Other *Staphylococcus* spp. found to cause persistence in a Norwegian study included *S. chromogenes, S. simulans, S. aureus, S. epidermidis, S. haemolyticus*, and *S. warneri* (Mørk et al., 2012). In contrast, a Belgian study found that when comparing NAS species, *S. xylosus* was more associated with persistent infection compared to transient IMI and *S. simulans* more transient than persistent (Supré et al., 2011). *Streptococcus uberis* IMI have been previously reported to persist, and subclinical infections were noted to have longer duration than clinical infections (Zadoks et al., 2003); it is possible the persistent *Strep. uberis* IMI observed in our study may conform to these past reported observations.

The persistence analysis was limited by the timeframe of the sampling period, so it was unknown if a given quarter was infected prior to 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. Identifying bacterial 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 NAS (Fry et al., 2014, Bernier Gosselin et al., 2019).

Prior research has indicated that SCC could be used diagnostically to identify dairy cows with and without IMI (Nyman et al., 2016). Our study used a longitudinal analysis to investigate whether SCC at first sampling could identify persistence of infection and found that initial SCC was associated with persistent IMI but was not predictive at the pathogen level. This information could help guide farmers to identify potential "costly" animals whose prolonged infections may result in future losses due to decreased milk production or increased SCC. Based on the results of this study, the utility of SCC to predict persistence of infection even absent significance at the specific pathogen level was not deemed useful because a post-hoc receiver operating characteristic curve showed the predicted SCC to be extremely low. Expanding the data collection pool may yield more statistically relevant findings at the pathogen level and should be considered for future research pursuit. Future studies involving dairies across a greater geographical area of the US might be more reflective of the national Jersey cattle population.

Overall, an interesting observation was that the average SCC was lower than expected for IMI-positive quarters. Some prior studies investigating IMI prevalence in primiparous cows indicated that Jerseys tended to be in a higher SCC range than Holsteins (Persson Waller et al., 2020). One study reported an 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 (Berry et al., 2007). One explanation for this 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). Further investigation would be needed to assess the relationship between milking quantity and SCC. Still, the breed-related discrepancy in average SCC has not been reflected consistently, as shown in a large-scale nationwide study that did not report significantly different average SCC between the 2 breeds (Capper and Cady, 2012).

The reported farm bulk tank SCC at sampling times were noticeably higher than the pathogen-specific predicted SCC values. A potential reason for our observed low SCC could be that detected organisms were present in milk samples but not causing infection and inflammation i.e. the cultured bacteria were contaminants from the skin or streak canal, which would havel owered the average SCC within pathogen grouping. Further, if the inoculum volume of milk was greater than the approximated quantity of 10µL, the sensitivity of microorganism detection would be increased, (reducing false negative results), and specificity decreased, (increasing false positive results).

Ideally, we would have evaluated farms with similar production systems to reduce farm differences as much as possible. Given that the scope of this research is not focused on assessment of mastitis risk factors, it is 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 microorganism populations and SCC associations at the quarter level.

A limitation of IMI detection in this study was that specific media for *Mycoplasma* growth was not used and hence any *Mycoplasma* spp. would have been overlooked as this bacterium requires special growth conditions (Bokma et al., 2019) that were not performed. Additionally, results may have been skewed due to failure of full

species identification. A number of IMI were only identifiable to the genus level, which may have resulted in under-representation of microorganisms of a given individual species. Genus-level identification limitations would also have contributed toward false identification of certain IMI as mixed (i.e. if 1 isolate was characterized to the species level and another only to the genus level, the sample would have been considered a mixed infection due to the presence of 2 distinct microorganisms).

The plate extraction MALDI-TOF method was used, which yields similar identification percentage to the tube extraction MALDI-TOF protocol, but the latter method could have been performed for instances when genus identification was unsuccessful (Barcelos et al., 2019). Other tests for microorganism identification, such as 16S rRNA sequencing, were not pursued. One study evaluating the diagnostic ability of MALDI-TOF in streptococcal identification deemed it a valuable modality with less expense and time utilization than 16s rRNA (Alnakip et al., 2020). Still, using a single protocol for microorganism identification could have resulted in misclassification error. Unidentified infections could also have resulted if the microorganism was not a part of the MALDI-TOF species library database. Ultimately, inability to accurately identify isolates would influence the overall IMI prevalence calculations by misclassification of data and over censoring of results.

When faced with the present-day challenges dairy producers must address, including rising feed costs, limited farming acreage, and increasing demand for more sustainable agriculture, Jerseys offer several advantages in efficiency and longevity when compared to their larger Holstein counterparts. Jerseys have been found to have longer production lives compared to other breeds (Hare et al., 2006) and may be more suitable

for meeting some of the future needs of the global food supply. Furthering our knowledge of this increasingly popular dairy breed will allow producers to capitalize on the environmental and consumer benefits Jerseys offer.

CONCLUSIONS

- Intramammary infection prevalence was successfully characterized in the enrolled Jersey herds. Non-*aureus* staphylococci, particularly *S. chromogenes* and *S. simulans*, were the most commonly identified organisms
- Pathogen prevalence varied among farms but *S. chromogenes*, *S. aureus*, and *Strep. dysgalactiae* were identified on all farms. *Staphylococcus chromogenes* was 1 of the top 2 most prevalent microorganisms isolated at each farm.
- Pathogen-specific associations with SCC revealed some significant differences among causes of IMI. Additionally, an interaction with days in milk was observed for some IMI.
- Predicted SCC associated with *S. haemolyticus*, *Bacillus* spp. and coliform infected quarters were not significantly different from quarters with no growth.
- First sampling SCC had a significant association with persistence but was not predictive of pathogen-specific IMI persistence.
- Overall, no major differences were found in this study from other reports in other dairy breeds. Similar IMI microorganism species were encountered in Jerseys and their associated SCC alterations approximated those described previously in the literature.

APPENDICES

Appendix 1. Detection of microorganisms at the quarter level from all milk samples collected from 4 Jersey dairy herds sampled once monthly for 3 consecutive months.

		HERD 1				HERD 2				HERD 3				HERD 4			
PATHOGEN	TOTAL	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3
No Significant Growth	5543	2470	804	861	805	946	330	332	284	614	202	211	201	1513	555	448	510
Acinetobacter indicus	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Aerococcus spp.	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Aerococcus viridans	2	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0
Arthrobacter gandavensis	3	0	0	0	0	0	0	0	0	3	1	0	2	0	0	0	0
Bacillus licheniformis	3	2	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0
Bacillus pumilus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus sonorensis	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus spp.	6	5	2	3	0	1	1	0	0	0	0	0	0	0	0	0	0
Brevibacillus borstelensis	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Brevibacterium luteolum	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Brevibacterium spp.	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Candida kefyr	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Candida krusei	4	0	0	0	0	4	2	1	1	0	0	0	0	0	0	0	0
Candida lusitaniae	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Candida rugosa	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Candida spp.	10	8	1	1	6	2	2	0	0	0	0	0	0	0	0	0	0
Candida tropicalis	3	3	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Citrobacter koseri	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium amycolatum	36	0	0	0	0	13	6	5	2	0	0	0	0	23	15	3	5

		HERD 1				HERD 2				HERD 3				HERD 4			
PATHOGEN	TOTAL	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3
Corynebacterium spp.	16	4	4	0	0	3	0	2	1	3	0	1	2	6	2	2	2
Corynebacterium ulcerans	4	4	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Corynebacterium xerosis	2	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0
Enterobacter xiangfangensis	2	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Enterococcus aquimarinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterococcus faecalis	4	4	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Enterococcus faecium	2	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0
Enterococcus hirae	2	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0
Escherichia coli	5	0	0	0	0	4	0	1	3	0	0	0	0	1	1	0	0
Globicatella spp.	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Glutamicibacter mysorens	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Janibacter spp.	3	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Kocuria carniphila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus garvieae	13	11	6	3	2	0	0	0	0	0	0	0	0	2	1	0	1
Lactococcus lactis	4	3	2	1	0	0	0	0	0	1	0	1	0	0	0	0	0
Lactococcus spp.	2	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Listeria monocytogenes	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Lysinibacillus sphaericus	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysinibacillus spp.	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paenibacillus spp.	3	0	0	0	0	2	2	0	0	1	0	0	1	0	0	0	0
Proteus hauseri	4	0	0	0	0	0	0	0	0	0	0	0	0	4	2	1	1
Proteus vulgaris	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Providencia rettgeri	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	10	4	2	1	1	1	1	0	0	1	1	0	0	4	1	2	1
Pseudomonas koreensis	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0

		HERD 1				HERD 2				HERD 3				HERD 4			
PATHOGEN	TOTAL	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3
Psychrobacter pasteurii	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Psychrobacter spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhodoccocus spp.	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Rhodococcus hoagii	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Rummeliibacillus spp.	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Serratia marcescens	16	4	1	2	1	11	8	2	1	1	0	1	0	0	0	0	0
Serratia ureilytica	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Staphylococcus agnetis/hyicus	5	1	1	0	0	1	1	0	0	1	1	0	0	2	0	1	1
Staphylococcus aureus	87	6	1	2	3	15	10	3	2	64	21	20	23	2	1	1	0
Staphylococcus auricularis	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Staphylococcus chromogenes	320	71	23	24	24	66	24	23	19	55	17	18	20	128	53	36	39
Staphylococcus devriesei	2	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0	0
Staphylococcus epidermidis	2	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1
Staphylococcus equorum	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Staphylococcus gallinarum	17	7	1	4	2	4	1	1	2	0	0	0	0	6	2	1	3
Staphylococcus haemolyticus	19	0	0	0	0	10	4	4	2	0	0	0	0	9	4	2	3
Staphylococcus nepalensis	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus sciuri	5	2	2	0	0	0	0	0	0	0	0	0	0	3	0	1	2
Staphylococcus simulans	202	0	0	0	0	1	0	0	1	0	0	0	0	201	72	66	63
Staphylococcus warneri	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Staphylococcus xylosus	12	0	0	0	0	3	1	1	1	0	0	0	0	9	3	3	3
Streptococcus agalactiae	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Streptococcus canis	7	0	0	0	0	1	1	0	0	6	2	1	3	0	0	0	0
Streptococcus dysgalactiae	36	17	7	7	3	2	0	0	2	8	1	2	5	9	2	6	1

		HERD 1				HERD 2				HERD 3				HERD 4			
PATHOGEN	TOTAL	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3	All	Visit 1	Visit 2	Visit 3
Streptococcus gallolyticus	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus lutetiensis	3	2	2	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Streptococcus spp.	13	8	2	1	5	2	0	2	0	0	0	0	0	3	1	1	1
Streptococcus uberis	22	14	5	6	3	7	2	3	2	1	0	1	0	0	0	0	0
Trichosporon asahii	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Weissella spp.	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Undetermined Identity	84	31	17	7	7	17	3	6	8	17	7	6	4	19	5	7	7
Mixed Infection	60	15	5	6	4	14	7	5	2	10	0	6	4	21	11	4	6
Contaminated	742	163	69	33	61	107	17	17	73	97	57	23	17	375	72	168	135
No Sample	194	69	15	27	27	26	10	9	7	37	8	7	22	62	20	28	14

APPENDIX 2. The following microorganisms were detected < 15 times and listed by herd (within-herd quantity noted in parenthesis if > 1 and * indicates microorganisms found in multiple herds). Example: *Bacillus licheniformis* was encountered twice in herd 1 and was also isolated at another farm.

Herd 1: *Aerococcus spp., *Bacillus licheniformis* (2), *Bacillus pumilus, Bacillus sonorensis,*Bacillus spp.* (5), *Candida* spp. (8), *Candida tropicalis* (3), *Corynebacterium ulcerans* (4), *Enterobacter xiangfangensis, *Enterococcus faecalis* (4), *Enterococcus faecium, Glutamicibacter mysorens, *Janibacter spp.* (4), *Kocuria carniphila, *Lactococcus garvieae* (11), *Lactococcus lactis* (3), *Lactococcus spp.* (2), *Lysinibacillus sphaericus, Lysinibacillus spp., Providencia rettgeri, *Pseudomonas aeruginosa* (4), *Staphylococcus hyicus* (2), *Staphylococcus sciuri* (2), *Staphylococcus nepalensis, Streptococcus gallolyticus, *Streptococcus lutetiensis* (2), *Streptococcus* spp. (8), *Trichosporon asahii.*

Herd 2: *Acinetobacter indicus, *Bacillus spp.***,** *Brevibacillus borstelensis, Brevibacterium luteolum, Brevibacterium spp., Candida kefyr, *Candida krusei* (5), *Candida lusitaniae, *Candida* spp. (2),*Enterobacter xiangfangensis, *Enterococcus hirae* (2), *Escherichia coli* (4), *Paenibacillus spp.* (2), *Pseudomonas aeruginosa, Pseudomonas koreensis, *Psychrobacter pasteurii, Psychrobacter spp., Rummeliibacillus spp., Serratia ureilytica, *Staphylococcus devriesei* (2), *Staphylococcus hyicus, *Staphylococcus xylosus* (3), *Streptococcus canis, *Streptococcus lutetiensis, *Streptococcus* spp. (2), *Weissella spp..*

Herd 3: *Arthrobacter gandavensis* (3), *Bacillus licheniformis, Candida rugosa, Enterococcus aquimarinus, Globicatella spp., *Lactococcus lactis, Listeria monocytogenes, *Paenibacillus spp., *Pseudomonas aeruginosa, Rhodoccocus spp.,

*Rhodococcus hoagie, *Staphylococcus agnetis, Staphylococcus warneri, Streptococcus agalactiae, *Streptococcus canis* (6).

Herd 4: **Aerococcus viridans* (2), *Citrobacter koseri, *Corynebacterium xerosis, *Enterococcus faecium* (2), *Escherichia coli* (2), *Lactococcus garvieae* (2), *Proteus hauseri* (4), *Proteus vulgaris, *Pseudomonas aeruginosa* (4), *Psychrobacter pasteurii* (2), *Staphylococcus agnetis* (2), *Staphylococcus auricularis, *Staphylococcus epidermidis* (2), *Staphylococcus equorum, *Staphylococcus sciuri* (3), *Staphylococcus xylosus* (9), **Streptococcus* spp. (3).

BIBLIOGRAPHY

Adkins, P. R. F., S. Dufour, J. N. Spain, M. J. Calcutt, T. J. Reilly, G. C. Stewart, and J. R. Middleton. 2018. Molecular characterization of non-aureus *Staphylococcus* spp. from heifer intramammary infections and body sites. J. Dairy Sci. 101:5388-5403. https://doi: 10.3168/jds.2017-13910.

Alnakip, M. E. A., N. R. Rhouma, E. N. Abd-Elfatah, M. Quintela-Baluja, K. Böhme, I. Fernández-No, M. A. Bayoumi, M. M. Abdelhafez, A. Taboada-Rodríguez, P. Calo-Mata, and J. Barros-Velázquez. 2020. Discrimination of major and minor streptococci incriminated in bovine mastitis by MALDI-TOF MS fingerprinting and 16S rRNA gene sequencing. Res. Vet. Sci. 132:426-438. https://doi: 10.1016/j.rvsc.2020.07.027.

Barcelos, M. M., L. Martins, R. C. Grenfell, L. Juliano, K. L. Anderson, M. V. Dos Santos, and J. L. Gonçalves. 2019. Comparison of standard and on-plate extraction protocols for identification of mastitis-causing bacteria by MALDI-TOF MS. Braz. J. Microbiol. 50:849-857. https://doi: 10.1007/s42770-019-00110-5.

Bernier Gosselin, V., S. Dufour, P. R. F. Adkins, S. Poock, P. Pithua, and J. R. Middleton. 2019. Longitudinal microbiological evaluation of subclinical non-*aureus* staphylococcal intramammary infections in a lentivirus-infected dairy goat herd. Vet. Microbiol. 230:156-163. https://doi: 10.3168/jds.S0022-0302(99)75393-2.

Berry, D. P., J. M. Lee, K. A. Macdonald, K. Stafford, L. Matthews, and J. R. Roche. 2007. Associations among body condition score, body weight, somatic cell count, and clinical mastitis in seasonally calving dairy cattle. J. Dairy Sci. 90:637-648. https://doi: 10.3168/jds.S0022-0302(07)71546-1.

Bexiga, R., M. G. Rato, A. Lemsaddek, T. Semedo-Lemsaddek, C. Carneiro, H. Pereira, D. J. Mellor, K. A. Ellis, and C. L. Vilela. 2014. Dynamics of bovine intramammary infections due to coagulase-negative staphylococci on four farms. J. Dairy Res. 81:208-214. https://doi: 10.1017/S0022029914000041.

Bobbo, T., P. L. Ruegg, G. Stocco, E. Fiore, M. Gianesella, M. Morgante, D. Pasotto, G. Bittante, and A. Cecchinato. 2017. Associations between pathogen-specific cases of subclinical mastitis and milk yield, quality, protein composition, and cheese-making traits in dairy cows. J. Dairy Sci. 100:4868-4883. https://doi: 10.3168/jds.2016-12353.

Bokma, J., B. Pardon, L. Van Driessche, L. Gille, P. Deprez, F. Haesebrouck, and F. Boyen. 2019. Optimizing identification of *Mycoplasma bovis* by MALDI-TOF MS. Res. Vet. Sci. 125:185-188. https://doi: 10.1016/j.rvsc.2019.06.010.

Cameron, M., H. W. Barkema, J. De Buck, S. De Vliegher, M. Chaffer, J. Lewis, and G. P. Keefe. 2017. Identification of bovine-associated coagulase-negative staphylococci by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a direct transfer protocol. J. Dairy Sci. 100:2137-2147. https://doi: 10.3168/jds.2016-12020.

Cameron, M., J. Perry, J. R. Middleton, M. Chaffer, J. Lewis, and G. P. Keefe. 2018. Short communication: Evaluation of MALDI-TOF mass spectrometry and a custom reference spectra expanded database for the identification of bovine-associated coagulase-negative staphylococci. J. Dairy Sci. 101:590-595. https://doi: 10.3168/jds.2017-13226.

Capper, J. L. and R. A. Cady. 2012. A comparison of the environmental impact of Jersey compared with Holstein milk for cheese production. J. Dairy Sci. 95:165-176. https://doi: 10.3168/jds.2011-4360.

Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, S. Naushad, S. De Vliegher, R. N. Zadoks, J. R. Middleton, S. Dufour, J. P. Kastelic, and H. W. Barkema. 2017a. Prevalence of non-*aureus* staphylococci species causing intramammary infections in Canadian dairy herds. J. Dairy Sci. 100:5592-5612. https://doi: 10.3168/jds.2016-12478.

Condas, L. A. Z., J. De Buck, D. B. Nobrega, D. A. Carson, J. P. Roy, G. P. Keefe, T. J. DeVries, J. R. Middleton, S. Dufour, and H. W. Barkema. 2017b. Distribution of non-*aureus* staphylococci species in udder quarters with low and high somatic cell count, and clinical mastitis. J. Dairy Sci. 100:5613-5627. https://doi: 10.3168/jds.2016-12479.

Council on Dairy Cattle Breeding. 2018. Council on Dairy Cattle Breeding Activity Report Oct 17/Sept 18. Accessed September 25, 2020. https://www.uscdcb.com/wp-content/uploads/2018/10/CDCB_Report_2018_final_web_REV2.pdf.

De Buck, J., V. Ha, S. Naushad, D. B. Nobrega, C. Luby, J. R. Middleton, S. De Vliegher, and H. W. Barkema. 2021. Non-*aureus* Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Front. Vet. Sci. 8:658031. https://doi: 10.3389/fvets.2021.658031.

De Haas, Y., H. W. Barkema, and R. F. Veerkamp. 2002. The effect of pathogen-specific clinical mastitis on the lactation curve for somatic cell count. J. Dairy Sci. 85:1314-1323. https://doi: 10.3168/jds.S0022-0302(02)74196-9.

De Visscher, A., S. Piepers, F. Haesebrouck, and S. De Vliegher. 2016. Intramammary infection with coagulase-negative staphylococci at parturition: Species-specific prevalence, risk factors, and effect on udder health. J. Dairy Sci. 99:6457-6469. https://doi: 10.3168/jds.2015-10458.

Dohoo, I. R., J. Smith, S. Andersen, D. F. Kelton, and S. Godden. 2011. Diagnosing intramammary infections: evaluation of definitions based on a single milk sample. J. Dairy Sci. 94:250-261. https://doi: 10.3168/jds.2010-3559.

Du, J., X. Wang, H. Luo, Y. Wang, X. Liu, and X. Zhou. 2018. Epidemiological investigation of non-*albicans Candida* species recovered from mycotic mastitis of cows in Yinchuan, Ningxia of China. BMC Vet. Res. 14(1):251. https://doi: 10.1186/s12917-018-1564-3.

Dworecka-Kaszak, B., A. Krutkiewicz, D. Szopa, M. Kleczkowski, and M. Biegańska. 2012. High prevalence of *Candida* yeast in milk samples from cows suffering from mastitis in poland. Scientific World Journal 2012:196347. https://doi: 10.1100/2012/196347.

Friman, M. J., M. H. Eklund, A. H. Pitkälä, P. J. Rajala-Schultz, and M. H. J. Rantala. 2019. Description of two *Serratia marcescens* associated mastitis outbreaks in Finnish dairy farms and a review of literature. Acta Vet. Scand. 61:54. https://doi: 10.1186/s13028-019-0488-7.

Fry, P. R., J. R. Middleton, S. Dufour, J. Perry, D. Scholl, and I. Dohoo. 2014. Association of coagulase-negative staphylococcal species, mammary quarter milk somatic cell count, and persistence of intramammary infection in dairy cattle. J. Dairy Sci. 97:4876-4885. https://doi: 10.3168/jds.2013-7657.

Gonçalves, J. L., R. I. Cue, B. G. Botaro, J. A. Horst, A. A. Valloto, and M. V. Santos. 2018. Milk losses associated with somatic cell counts by parity and stage of lactation. J. Dairy Sci. 101:4357-4366. https://doi: 10.3168/jds.2017-13286.

Gonçalves, J. L., C. Kamphuis, H. Vernooij, J. P. Araújo, Jr., R. C. Grenfell, L. Juliano, K. L. Anderson, H. Hogeveen, and M. V. Dos Santos. 2020. Pathogen effects on milk yield and composition in chronic subclinical mastitis in dairy cows. Vet. J. 262:105473. https://doi: 10.1016/j.tvjl.2020.105473.

Gonçalves, J. L., T. Tomazi, J. R. Barreiro, D. C. Beuron, M. A. Arcari, S. H. Lee, C. M. Martins, J. P. Araújo Junior, and M. V. dos Santos. 2016. Effects of bovine subclinical mastitis caused by *Corynebacterium* spp. on somatic cell count, milk yield and composition by comparing contralateral quarters. Vet. J. 209:87-92. https://doi: 10.1016/j.tvjl.2015.08.009.

Gonzalez-Peña, D., N. Vukasinovic, J. J. Brooker, C. A. Przybyla, A. Baktula, and S. K. DeNise. 2020. Genomic evaluation for wellness traits in US Jersey cattle. J. Dairy Sci. 103:1735-1748. https://doi: 10.3168/jds.2019-16903.

Halasa, T., K. Huijps, O. Østerås, and H. Hogeveen. 2007. Economic effects of bovine mastitis and mastitis management: a review. Vet. Q. 29:18-31. https://doi: 10.1080/01652176.2007.9695224.

Halasa, T., M. Nielen, A. P. De Roos, R. Van Hoorne, G. de Jong, T. J. Lam, T. van Werven, and H. Hogeveen. 2009. Production loss due to new subclinical mastitis in

Dutch dairy cows estimated with a test-day model. J. Dairy Sci. 92:599-606. https://doi: 10.3168/jds.2008-1564.

Hare, E., H. D. Norman, and J. R. Wright. 2006. Survival rates and productive herd life of dairy cattle in the United States. J. Dairy Sci. 89:3713-3720. https://doi: 10.3168/jds.S0022-0302(06)72412-2.

Heikkilä, A. M., E. Liski, S. Pyörälä, and S. Taponen. 2018. Pathogen-specific production losses in bovine mastitis. J. Dairy Sci. 101:9493-9504. https://doi: 10.3168/jds.2018-14824.

International Dairy Federation. 2013. Guidelines for the use and interpretation of bovine milk somatic cell counts (SCC) in the dairy industry. Accessed May 21, 2022. https://www.fil-idf.org/wp-content/uploads/woocommerce_uploads/2013/03/B466-2013Guidelines-for-the-use-and-interpretation-of-bovine-milk-SCC-in-the-dairy-industry.CAT_-lmbdpy.pdf.

Jones, T. O. and P. C. Turnbull. 1981. Bovine mastitis caused by *Bacillus cereus*. Vet. Rec. 108:271-274. https://doi: 10.1136/vr.108.13.271.

Keane, O. M. 2019. Symposium review: Intramammary infections-Major pathogens and strain-associated complexity. J. Dairy Sci. 102:4713-4726. https://doi: 10.3168/jds.2018-15326.

Liang, D., L. M. Arnold, C. J. Stowe, R. J. Harmon, and J. M. Bewley. 2017. Estimating US dairy clinical disease costs with a stochastic simulation model. J. Dairy Sci. 100:1472-1486. https://doi: 10.3168/jds.2016-11565.

Lücken, A., N. Wente, Y. Zhang, S. Woudstra, and V. Krömker. 2021. Corynebacteria in Bovine Quarter Milk Samples-Species and Somatic Cell Counts. Pathogens 10. https://doi: 10.3390/pathogens10070831.

Mahmmod, Y. S., I. C. Klaas, L. Svennesen, K. Pedersen, and H. Ingmer. 2018. Communications of *Staphylococcus aureus* and non-*aureus* Staphylococcus species from bovine intramammary infections and teat apex colonization. J. Dairy Sci. 101:7322-7333. https://doi: 10.3168/jds.2017-14311.

Manuelian, C. L., M. Penasa, G. Visentin, A. Zidi, M. Cassandro, and M. De Marchi. 2018. Mineral composition of cow milk from multibreed herds. Anim. Sci. J. 89:1622-1627. https://doi: 10.1111/asj.13095.

Martins, L., M. M. Barcelos, R. I. Cue, K. L. Anderson, M. V. D. Santos, and J. L. Gonçalves. 2020. Chronic subclinical mastitis reduces milk and components yield at the cow level. J. Dairy Res. 87: 298-305. https://doi: 10.1017/S0022029920000321.

Middleton, J. R., L. K. Fox, G. Pighetti, and C. Petersson-Wolfe. 2017. Laboratory Handbook on Bovine Mastitis. 3rd ed. National Mastitis Council, New Prague, MN.

Milanov, D., B. Prunic, M. Velhner, and J. Bojkovski. 2014. Diagnosis of Yeast Mastitis in Dairy Cows. LUCRĂRI ŞTIINŢIFICE MEDICINĂ VETERINARĂ. 47:56-64.

Mørk, T., H. J. Jørgensen, M. Sunde, B. Kvitle, S. Sviland, S. Waage, and T. Tollersrud. 2012. Persistence of staphylococcal species and genotypes in the bovine udder. Vet. Microbiol. 159:171-180. https://doi: 10.1016/j.vetmic.2012.03.034.

Nyman, A. K., U. Emanuelson, and K. P. Waller. 2016. Diagnostic test performance of somatic cell count, lactate dehydrogenase, and N-acetyl-β-D-glucosaminidase for detecting dairy cows with intramammary infection. J. Dairy Sci. 99:1440-1448. https://doi: 10.3168/jds.2015-9808.

Paape, M. J., B. Poutrel, A. Contreras, J. C. Marco, and A. V. Capuco. 2001. Milk Somatic Cells and Lactation in Small Ruminants. J. Dairy Sci. 84: E237-E244. https://doi.org/10.3168/jds.S0022-0302(01)70223-8.

Pantoja, J. C., C. Hulland, and P. L. Ruegg. 2009. Dynamics of somatic cell counts and intramammary infections across the dry period. Prev. Vet. Med. 90:43-54. https://doi: 10.1016/j.prevetmed.2009.03.012.

Persson Waller, K., Å. Lundberg, and A. K. Nyman. 2020. Udder health of early-lactation primiparous dairy cows based on somatic cell count categories. J. Dairy Sci. 103:9430-9445. https://doi: 10.3168/jds.2020-18346.

Piessens, V., E. Van Coillie, B. Verbist, K. Supré, G. Braem, A. Van Nuffel, L. De Vuyst, M. Heyndrickx, and S. De Vliegher. 2011. Distribution of coagulase-negative Staphylococcus species from milk and environment of dairy cows differs between herds. J. Dairy Sci. 94:2933-2944. https://doi: 10.3168/jds.2010-3956.

Pitkälä, A., M. Haveri, S. Pyörälä, V. Myllys, and T. Honkanen-Buzalski. 2004. Bovine mastitis in Finland 2001--prevalence, distribution of bacteria, and antimicrobial resistance. J. Dairy Sci. 87:2433-2441. https://doi: 10.3168/jds.S0022-0302(04)73366-4.

Potter, T. L., C. Arndt, and A. N. Hristov. 2018. Short communication: Increased somatic cell count is associated with milk loss and reduced feed efficiency in lactating dairy cows. J. Dairy Sci. 101:9510-9515. https://doi: 10.3168/jds.2017-14062.

Reyher, K. K., D. Haine, I. R. Dohoo, and C. W. Revie. 2012. Examining the effect of intramammary infections with minor mastitis pathogens on the acquisition of new

intramammary infections with major mastitis pathogens--a systematic review and meta-analysis. J. Dairy Sci. 95:6483-6502. https://doi: 10.3168/jds.2012-5594.

Rollin, E., K. C. Dhuyvetter, and M. W. Overton. 2015. The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool. Prev. Vet. Med. 122:257-264. https://doi: 10.1016/j.prevetmed.2015.11.006.

Rowe, S. M., S. M. Godden, E. Royster, J. Timmerman, B. A. Crooker, and M. Boyle. 2019. Cross-sectional study of the relationships among bedding materials, bedding bacteria counts, and intramammary infection in late-lactation dairy cows. J. Dairy Sci. 102:11384-11400. https://doi: 10.3168/jds.2019-17074.

Ruegg, P. L. 2017. A 100-Year Review: Mastitis detection, management, and prevention. J. Dairy Sci. 100:10381-10397. https://doi: 10.3168/jds.2017-13023.

Schukken, Y., M. Chuff, P. Moroni, A. Gurjar, C. Santisteban, F. Welcome, and R. Zadoks. 2012. The "other" gram-negative bacteria in mastitis: *Klebsiella, Serratia,* and more. Vet. Clin. North Am. Food Anim. Pract. 28:239-256. https://doi: 10.1016/j.cvfa.2012.04.001.

Schwarz, D., U. S. Diesterbeck, K. Failing, S. König, K. Brügemann, M. Zschöck, W. Wolter, and C. P. Czerny. 2010. Somatic cell counts and bacteriological status in quarter

foremilk samples of cows in Hesse, Germany--a longitudinal study. J. Dairy Sci. 93:5716-5728. https://doi: 10.3168/jds.2010-3223.

Schiefer, B., K. R. Macdonald, G. G. Klavano, and A. A. van Dreumel. 1976. Pathology of *Bacillus cereus* mastitis in dairy cows. Can. Vet. J. 17:239-243.

Sewalem, A., F. Miglior, G. J. Kistemaker, and B. J. Van Doormaal. 2006. Analysis of the relationship between somatic cell score and functional longevity in Canadian dairy cattle. J. Dairy Sci. 89:3609-3614. https://doi: 10.3168/jds.S0022-0302(06)72400-6.

Stocco, G., C. Cipolat-Gotet, V. Gasparotto, A. Cecchinato, and G. Bittante. 2018. Breed of cow and herd productivity affect milk nutrient recovery in curd, and cheese yield, efficiency and daily production. Animal 12:434-444. https://doi: 10.1017/S1751731117001471.

Sumon, S., M. S. Parvin, M. A. Ehsan, and M. T. Islam. 2020. Dynamics of somatic cell count and intramammary infection in lactating dairy cows. J. Adv. Vet. Anim. Res. 7:314-319. https://doi: 10.5455/javar.2020.g423.

Sun, M., J. Gao, T. Ali, D. Yu, S. Zhang, S. U. Khan, S. Fanning, and B. Han. 2017. Characteristics of *Aerococcus viridans* isolated from bovine subclinical mastitis and its effect on milk SCC, yield, and composition. Trop. Anim. Health Prod. 49:843-849. https://doi: 10.1007/s11250-017-1271-2.

Supré, K., F. Haesebrouck, R. N. Zadoks, M. Vaneechoutte, S. Piepers, and S. De Vliegher. 2011. Some coagulase-negative Staphylococcus species affect udder health more than others. J. Dairy Sci. 94:2329-2340. https://doi: 10.3168/jds.2010-3741.

Taponen, S., J. Koort, J. Björkroth, H. Saloniemi, and S. Pyörälä. 2007. Bovine intramammary infections caused by coagulase-negative staphylococci may persist throughout lactation according to amplified fragment length polymorphism-based analysis. J. Dairy Sci. 90:3301-3307. https://doi: 10.3168/jds.2006-860.

Tenhagen, B. A., G. Köster, J. Wallmann, and W. Heuwieser. 2006. Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. J. Dairy Sci. 89:2542-2551. https://doi: 10.3168/jds.S0022-0302(06)72330-X.

Thorberg, B. M., M. L. Danielsson-Tham, U. Emanuelson, and K. Persson Waller. 2009. Bovine subclinical mastitis caused by different types of coagulase-negative staphylococci. J. Dairy Sci. 92:4962-4970. https://doi: 10.3168/jds.2009-2184.

Todhunter, D. A., K. L. Smith, and J. S. Hogan. 1991. Serratia species isolated from bovine intramammary infections. J. Dairy Sci. 74:1860-1865. https://doi: 10.3168/jds.S0022-0302(91)78351-3.

Tomazi, T., J. L. Gonçalves, J. R. Barreiro, M. A. Arcari, and M. V. dos Santos. 2015. Bovine subclinical intramammary infection caused by coagulase-negative staphylococci increases somatic cell count but has no effect on milk yield or composition. J. Dairy Sci. 98:3071-3078. https://doi: 10.3168/jds.2014-8466.

Valckenier, D., S. Piepers, A. De Visscher, R. M. Bruckmaier, and S. De Vliegher. 2019. Effect of intramammary infection with non-aureus staphylococci in early lactation in dairy heifers on quarter somatic cell count and quarter milk yield during the first 4 months of lactation. J. Dairy Sci. 102:6442-6453. https://doi: 10.3168/jds.2018-15913.

Valckenier, D., S. Piepers, A. De Visscher, and S. De Vliegher. 2020. The effect of intramammary infection in early lactation with non-*aureus* staphylococci in general and Staphylococcus chromogenes specifically on quarter milk somatic cell count and quarter milk yield. J. Dairy Sci. 103:768-782. https://doi: 10.3168/jds.2019-16818.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, and S. De Vliegher. 2014. Invited review: effect, persistence, and virulence of coagulase-negative Staphylococcus species associated with ruminant udder health. J. Dairy Sci. 97:5275-5293. https:// doi: 10.3168/jds.2013-7775.

Vanderhaeghen, W., S. Piepers, F. Leroy, E. Van Coillie, F. Haesebrouck, and S. De Vliegher. 2015. Identification, typing, ecology and epidemiology of coagulase negative

staphylococci associated with ruminants. Vet. J. 203:44-51. https://doi: 10.1016/j.tvjl.2014.11.001.

Wattenburger, K., R. Schmidt, L. Placheta, J. R. Middleton, and P. R. F. Adkins. 2020. Evaluation of 4 different teat disinfection methods prior to collection of milk samples for bacterial culture in dairy cattle. J. Dairy Sci. 103:4579-4587. https://doi: 10.3168/jds.2019-17338.

Wells, S. and S. Ott. 1998. The current milk quality in the US? Pages 10-18 in 1998 National Mastitis Council Annual Meeting Proceedings. National Mastitis Council.

White, S. L., G. A. Benson, S. P. Washburn, and J. T. Green, Jr. 2002. Milk production and economic measures in confinement or pasture systems using seasonally calved Holstein and Jersey cows. J. Dairy Sci. 85:95-104. https://doi: 10.3168/jds.S0022-0302(02)74057-5.

Zadoks, R. N., B. E. Gillespie, H. W. Barkema, O. C. Sampimon, S. P. Oliver, and Y. H. Schukken. 2003. Clinical, epidemiological and molecular characteristics of *Streptococcus uberis* infections in dairy herds. Epidemiol. Infect. 130:335-349. https://doi: 10.1017/s0950268802008221.

Zigo, F., Z. Farkašová, J. Výrostková, I. Regecová, S. Ondrašovičová, M. Vargová, N. Sasáková, E. Pecka-Kielb, Š. Bursová, and D. S. Kiss. 2022. Dairy Cows' Udder

Pathogens and Occurrence of Virulence Factors in Staphylococci. Animals (Basel) 12:470. https://doi: 10.3390/ani12040470.

VITA

Samantha Haw grew up in the San Francisco Bay Area, where she first became exposed to livestock through 4-H. She attained her undergraduate education at Dartmouth College, graduating with a Bachelor of Arts in Biology (area of concentration Animal Biology and Epidemiology). Samantha next attended veterinary school at the University of California, Davis, where she received her Doctor of Veterinary Medicine degree. She served as a veterinary associate in private mixed animal practice in Northern California for 4 years and completed an internship in Food Animal Medicine and Surgery at The Ohio State University prior to residency. Samantha is completing her third and final year of Large Animal Internal Medicine (Food Animal emphasis) residency with a combined Master of Science in Biomedical Sciences (emphasis in Veterinary Medicine and Surgery) degree program at the University of Missouri. Her research work has focused on subclinical mastitis in Jersey cows.