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Distribution of *Lactococcus* spp. in New York State dairy farms and the association of somatic cell count resolution and bacteriological cure in clinical mastitis samples

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ABSTRACT

We investigated the distribution of pathogenic non-*agalactiae* gram-positive, catalase-negative cocci (GPCN) in a convenience sample of New York State dairy farms. Our primary objective with the clinical mastitis (CM) GPCN samples was to evaluate somatic cell count (SCC) resolution and bacteriological cure of *Streptococcus dysgalactiae* or *Streptococcus uberis* versus *Lactococcus lactis* or *Lactococcus garvieae* in cows that received an approved intramammary treatment. In phase I, we assessed the distribution of the GPCN and SCC resolution. In phase II, we evaluated the SCC resolution and bacteriological cure in CM samples from the 4 farms with the highest prevalence of *L. lactis* or *L. garvieae* in phase I. In phase I, 8,868 CM and subclinical mastitis (SCM) milk samples were received from 143 farms. The GPCN samples identified by culture were confirmed with MALDI-TOF. From the 473 MALDI-TOF-confirmed GPCN samples, 155 were *S. dysgalactiae* (33%); 150, *S. uberis* (32%); 112, *L. lactis* (24%); 16, *L. garvieae* (3%); and 40, other GPCN (8%). From these, 277 were CM samples and 127 were eligible for the evaluation of SCC resolution, which was defined as SCC ≤200,000 cells/mL in a composite sample 15 to 60 d post-diagnosis. The odds of SCC resolution in CM samples was evaluated with multivariable logistic regression, and the odds were 6.1 [95% confidence interval (CI):2.7–13.9] times higher for *S. dysgalactiae* or *S. uberis* compared with *L. lactis* or *L. garvieae*. In phase II, a total of 1,662 CM and SCM samples were evaluated with microbiological methods as in phase I, of which 211 samples were confirmed by MALDI-TOF: 39% were *S. dysgalactiae* (n = 61) and *S. uberis* (n = 21); 55%, *L. lactis* (n = 114) and *L. garvieae* (n = 2); and 6%, other GPCN (n = 13). In total, 168 CM samples were eligible for analysis and 118 were included in the final SCC resolution model. Similar statistical methods as in phase I were performed, and the odds of SCC resolution were 2.4 (95% CI: 1.1–5.5) times higher for *S. dysgalactiae* or *S. uberis* compared with *L. lactis* or *L. garvieae*. Bacteriological cure was defined as having a different or negative culture on a quarter sample taken 14 to 28 d after initial diagnosis. The odds of bacteriological cure (n = 121) were 8.0 (95% CI: 2.5–25.6) times higher for *S. dysgalactiae* or *S. uberis* compared with *L. lactis* or *L. garvieae*. Differences in SCC resolution and bacteriological cure between these groups may dictate a different management approach.

Key words: gram-positive, catalase-negative cocci, clinical mastitis, MALDI-TOF, *Lactococcus* spp.

INTRODUCTION

Mastitis is an expensive disease affecting all farms in the dairy industry. It can cost more than $444 per clinical mastitis (**CM**) case (Rollin et al., 2015) and result in a 2 to 5% loss of 305-d milk in the average lactation per subclinical mastitis (**SCM**) case (Hagnestam-Nielsen et al., 2009). Both SCM and CM can cause an increase in SCC above 200,000 cells/mL (Dohoo et al., 1991), which negatively affects milk production, milk quality, and access to milk quality premiums (Hand et al., 2012a,b). Dairy farmers use a variety of prevention and treatment strategies to reduce the risk of mastitis in their cows (Huijps et al., 2010) to maintain milk production, decrease SCC, improve animal well-being, and increase income. One common strategy has been to treat all CM cases with intramammary (**IMM**) antimicrobials (Pol and Ruegg, 2007). However, research has raised questions about the economic viability of this approach, noting that pathogen-specific treatment targeting bacteria more likely to respond to IMM antimicrobials can decrease the treatment and milk-discard

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costs (MacDonald et al., 2011). Farms that adopted the strategy to use IMM antimicrobials in CM cases associated with gram-positive bacterial growth and not treat those with either gram-negative or no growth did not see a difference in cure rates (Keefe et al., 2010) or other CM-relevant outcomes such as recurrence, days to clinical cure, culling, or milk production (Lago et al., 2011a,b; Vasquez et al., 2017). As the focus of IMM antimicrobials narrows to gram-positive pathogens in CM samples (Oliver et al., 2004), understanding the distribution of these pathogens is a priority.

Recent research on the prevalence of mastitis pathogens at 5 farms in New York State (Hertl et al., 2014) reported that *Streptococcus* spp. represented the most common pathogen type associated with CM, with a prevalence of 22% of first cases, 18% of second cases, and 14% of third cases in a cow's lactation. Vasquez et al. (2016, 2017) also reported a high proportion of *Streptococcus* spp. in CM samples in New York dairy farms. This distribution is not unique to New York, and streptococci are commonly isolated from cow's milk with CM in Belgium (Verbeke et al., 2014), New Zealand (McDougall, 2003), Australia (Shum et al., 2009), Canada (Olde Riekerink et al., 2008), Italy (Ospina et al., 2016), and the United Kingdom (Bradley et al., 2007).

Although identification of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* is very accurate with standard microbiological methods (Wyder et al., 2011; Raemy et al., 2013), common biochemical tests cannot easily differentiate *Streptococcus* spp. from the remaining non-*agalactiae* gram-positive, catalase-negative cocci (**GPCN**), including species in the genera *Lactococcus*, *Enterococcus*, and *Aerococcus*. Consequently, these bacteria are misidentified 31 to 77% of the time (Fortin et al., 2003). Although the frequency of infection can vary between herds, GPCN can account for as much as 45% of IMM infections (Todhunter et al., 1995; Jones and Swisher, 2009; Hertl et al., 2014). In milk samples from CM and SCM cases, *Lactococcus lactis*, *Aerococcus viridans*, and *Streptococcus parauberis* pathogens were also difficult to differentiate phenotypically from other *Streptococcus* spp., indicating that these pathogens are likely to be misidentified and unreported as a potential cause of CM and SCM cases (Werner et al., 2014). Previous reports evaluating the prevalence of *Lactococcus* spp. found low levels of infection in SCM samples (3%, Haguingan et al., 2010; 7%, Devriese et al., 1999) and on a whole population level (0.9%, Wyder et al., 2011). As a result, these less common pathogens, such as *Lactococcus* spp., have been frequently grouped together as "*Streptococcus* species" or are misidentified as *Streptococcus uberis*, making it difficult to assess the significance of these unique organisms (Devriese et al., 1999; Fortin et al., 2003; Odierno et al., 2006). Compared with phenotypic methods and 16S rRNA sequencing, newer technology, such as MALDI-TOF (Randall et al., 2015), has been used to confirm the identification of many of these GPCN. Furthermore, *Lactococcus* spp. are common pathogens on some farms (Plumed-Ferrer et al., 2013; Werner et al., 2014; Plumed-Ferrer et al., 2015; Rodrigues et al., 2016), warranting additional research into these organisms.

In the current study, our objectives were (1) to describe the distribution of GPCN in CM and SCM milk samples based on MALDI-TOF identification in a convenience sample of New York dairy farms; (2) to compare DHIA SCC resolution for CM samples with *S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae* in CM samples that received an approved IMM antimicrobial; and (3) to evaluate SCC resolution and bacteriological cure in CM samples that received an approved IMM mastitis treatment and had *S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae* at farms with a high prevalence of *Lactococcus* spp.

MATERIALS AND METHODS

Sample Collection

Aseptic Milk Samples. Milk samples were aseptically collected by trained on-farm personnel or Quality Milk Production Services (**QMPS**) at Cornell University College of Veterinary Medicine staff and kept cool (1°C) or frozen (−20°C) until reaching either the QMPS laboratory in Canton, NY, or the Veterinary Clinic in Lowville, NY. These samples were then processed through MALDI-TOF for confirmation. This observational data set consisted of a convenience sample of quarter and composite milk samples from both CM and SCM cases as identified by farm personnel. The CM samples were collected from cows identified by farm personnel as having abnormal milk (e.g., clots, watery milk, blood), with or without changes to the udder. All cows with CM received an approved IMM antimicrobial based on farm protocols. The SCM samples included fresh cow screening, cows sampled due to high SCC, and whole herd sampling performed by QMPS technicians.

Composite SCC Sample Collection and Evaluation. Farms were enrolled in routine whole herd DHIA testing. Briefly, on test day, trained DHIA technicians obtained about 40 to 75 mL of individual composite milk samples at the time of milking from a portable metered sampler that is verified annually. The milk sample

was preserved with DHI milk preservative, which has an active ingredient of 18% 2-bromo-2- nitropropane-1, 3-diol (Bronopol-Boots, National DHIA, Columbus, OH), at the mandated 2.05 μL of preservative per 40 to 75 mL of milk sample. The samples were kept at ambient temperature or refrigerated to ensure they were not exposed to temperature extremes. The milk samples were then tested for SCC at DHIA (Dairy One, Ithaca, NY) using flow cytometry (Fossomatic, Foss, Hillerød, Denmark). Milk samples collected 15 to 60 d after CM diagnosis were included in the analysis.

Pathogen Identification

A sterile 15-cm wood-handled cotton swab (Puritan Medical Products Co., Guilford, ME; cotton swab dimensions 16 by 5 mm) was used to take the milk sample from the vial and used to streak one half of a trypticase soy agar plate containing 5% sheep blood and 0.1% esculin (Hardy Diagnostics, Santa Maria, CA) with approximately 30 μL of milk. Plates were incubated aerobically at 37°C for 24 h (National Mastitis Council, 2017). At 24 h, initial phenotypic identification based on colony morphology was performed to select for presumptive GPCN. Plates with at least 3 GPCN colonies were included in additional evaluation. An esculin hydrolysis reaction was evaluated. Esculin-negative colonies were further evaluated with Lancefield grouping PathoDx group C (Inzana and Iritani, 1989) and Christie–Atkins–Munch-Petersen factor test if necessary. Esculin-positive colonies were inoculated on bile esculin medium (Becton, Dickinson and Company, Sparks, MD). A single representative colony, excluding *S. agalactiae*, from the original plates was replated on blood agar and incubated for 24 h, after which a representative colony was evaluated with MALDI-TOF (Randall et al., 2015). The MALDI-TOF evaluation was done at the Ithaca QMPS laboratory. A log (score) $\geq$ 1.7 was the threshold for the genus-level identification and a log (score) of $\geq$2.0 was the set as the threshold for a match at the species level. Only samples identified to the species level were retained for further analysis.

Statistical Analysis

All data were compiled in Excel software (Microsoft Corp., Santa Rosa, CA) and imported into SAS 9.4 (SAS Institute Inc., Cary, NC) for statistical analysis. Three 3 models were evaluated in this prospective cohort study.

In phase I, the distribution of pathogenic GPCN in CM and SCM was described and the association between SCC resolution and pathogen group was evaluated in CM samples. In phase II, the distribution of pathogenic GPCN in CM samples from selected herds was also described. Two independent models were constructed to evaluate SCC resolution and bacteriological cure in CM samples. The risk difference between the unexposed (samples with *S. dysgalactiae* or *S. uberis*) versus the exposed (samples with *L. lactis* or *L. garvieae*) was estimated by subtracting 2 proportions. The proportions consisted of the number of samples with SCC resolution in phase I and II and bacteriological cure in phase II, each divided by the total number of samples from the exposed and exposed groups, respectively.

In these prospective cohort studies, cows entered the study when they were diagnosed with CM. They were then followed forward in time, and the association between the outcome of interest (i.e., SCC resolution or bacteriological cure) and the exposure (i.e., diagnosis with *S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae*) was evaluated. Based on a DHIA composite milk sample, collected 15 to 60 d after CM diagnosis, the SCC resolution (in both phase I and II) was defined if SCC was $\leq$200,000 cells/mL. Bacteriological cure (in phase II) was defined as a follow-up sample collected 14 to 28 d after CM diagnosis with the culture result being negative or showing the presence of a different pathogen. Throughout the study, the farm did not receive information regarding pathogen diagnosis before treatment, thus minimizing potential for systematic treatment bias based on pathogen.

Phase I Inclusion Criteria. To be included in the final analysis of phase I, the following criteria needed to be met: initial CM quarter-level sample, cow present for a follow-up DHIA sample between 15 and 60 d after CM diagnosis, and record of the cow having received an approved IMM antimicrobial treatment. Of the 277 CM samples available for analysis, the following were excluded: 49 were dropped because the original samples were composite samples; 76 were missing information about when the DHIA sample was collected or missing DHIA data; 5 were collected less than 2 wk after CM diagnosis; 9 were missing treatment information; and 11 contained a pathogen that was not one of the top pathogens (i.e., *S. dysgalactiae*, *S. uberis*, *L. lactis*, or *L. garvieae*). Thus, 127 samples from 10 farms were available for final analysis of SCC resolution in phase I (Figure 1).

Phase II Inclusion Criteria for SCC Resolution. To be included in the final analysis in the evaluation of SCC resolution in phase II, the following criteria needed to be met: initial CM quarter-level sample, cow present for a follow-up DHIA sample between 15 and 60 d after CM diagnosis, and record of the cow having received an approved IMM antimicrobial treatment.

Of the 168 cows sampled for CM, the following were excluded from the final analysis: 10 did not include one of thetwo pathogen groups (*S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae*), 6 initial CM milk samples were not quarter samples, 8 did not have IMM treatment information, and 26 were missing DHIA data. Thus, 118 samples were available for SCC resolution analysis in phase II (Figure 2).

Phase II Inclusion Criteria for Bacteriological Cure. To be included in the final analysis for bacteriological cure, the following criteria needed to be met: initial CM quarter-level sample, cow present for a follow-up quarter-level sample 14 to 28 d after CM diagnosis, and record of the cow having received an approved IMM antimicrobial treatment. From the 168 samples from cows with CM identified in phase II, the following were excluded from analysis: 10 did not include one of the top 2 pathogen groups, 6 did not originate from quarter samples, 8 did not have IMM treatment information (either none or unknown), and 23 did not have information about an outcome. Thus, 121 samples were eligible for bacteriological cure (Figure 2).

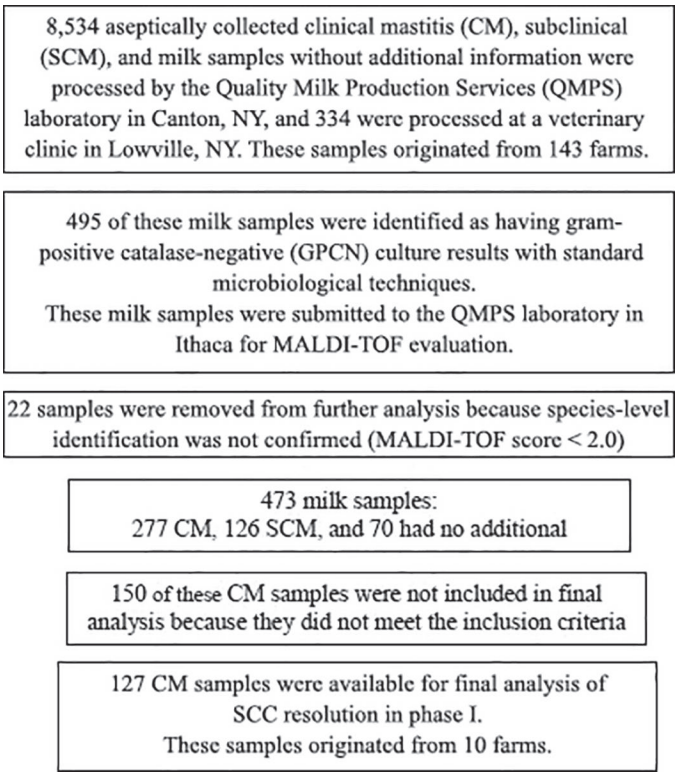

Figure 1. Flow diagram of samples included in final analysis of SCC resolution model in phase I (May to November 2014).

Models

The GLIMMIX procedure (SAS v. 9.4) was used to model the odds of SCC resolution in phase I and the independent odds of SCC resolution and bacteriological cure in phase II. In all models, a manual backward stepwise elimination strategy with farm as a random effect was used. Variables and interactions starting with $P > 0.25$ were eliminated, until only covariates with $P \leq 0.05$ remained in the final model. The following continuous variables were categorized to facilitate interpretation: parity, DIM at CM diagnosis, and number of days from CM diagnosis to DHIA testing. Parity was categorized into 2 groups: lactation = 1 and lactation ≥2. The DIM at CM diagnosis was categorized into 3 groups: early (1–30 DIM), mid lactation (31–180 DIM), and late lactation (>180 DIM). The number of days from CM diagnosis to DHIA testing was categorized into 3 groups: 15 to 30 d, 31 to 45 d, and 46 to 60 d. This variable was included only in the SCC resolution models.

SCC Resolution. In phases I and II, the initial multivariable models included pathogen group (*S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae*), parity group, DIM at diagnosis category, number of days from CM diagnosis to DHIA testing category, and the following interactions: pathogen group × parity group, and pathogen group × number of days from CM diagnosis to DHIA testing category.

In phase I, 127 samples were eligible for analysis. Parity groups 1 and 2 had 34 and 93 cows, respectively. Twenty-two cows were in early lactation, 46 were in mid lactation, and 59 were in late lactation. The number of days from CM to DHIA testing included 47 cows sampled at 15 to 30 d, 42 at 31 to 45 d, and 38 at 46 to 60 d. In phase I, the final model included only pathogen group (*S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae*) and farm as a random effect.

In phase II, 118 samples were eligible for analysis. Parity groups 1 and 2 had 20 and 98 cows, respectively. Eleven cows were in early lactation, 58 were in mid lactation, and 49 were in late lactation. The number of days from CM to DHIA testing included 64 cows sampled at 15 to 30 d, 40 at 31 to 45 d, and 14 at 46 to 60 d. In phase II, the final model evaluating SCC resolution included only the pathogen group (*S. dysgalactiae* or *S. uberis* versus *L. lactis* or *L. garvieae*) and farm as a random effect.

Bacteriological Cure. For the bacteriological cure model, the initial model included pathogen group, DIM-at-diagnosis group, parity group, and the interaction of pathogen group × parity group. Among the 121 samples available for analysis, 28 cows were in

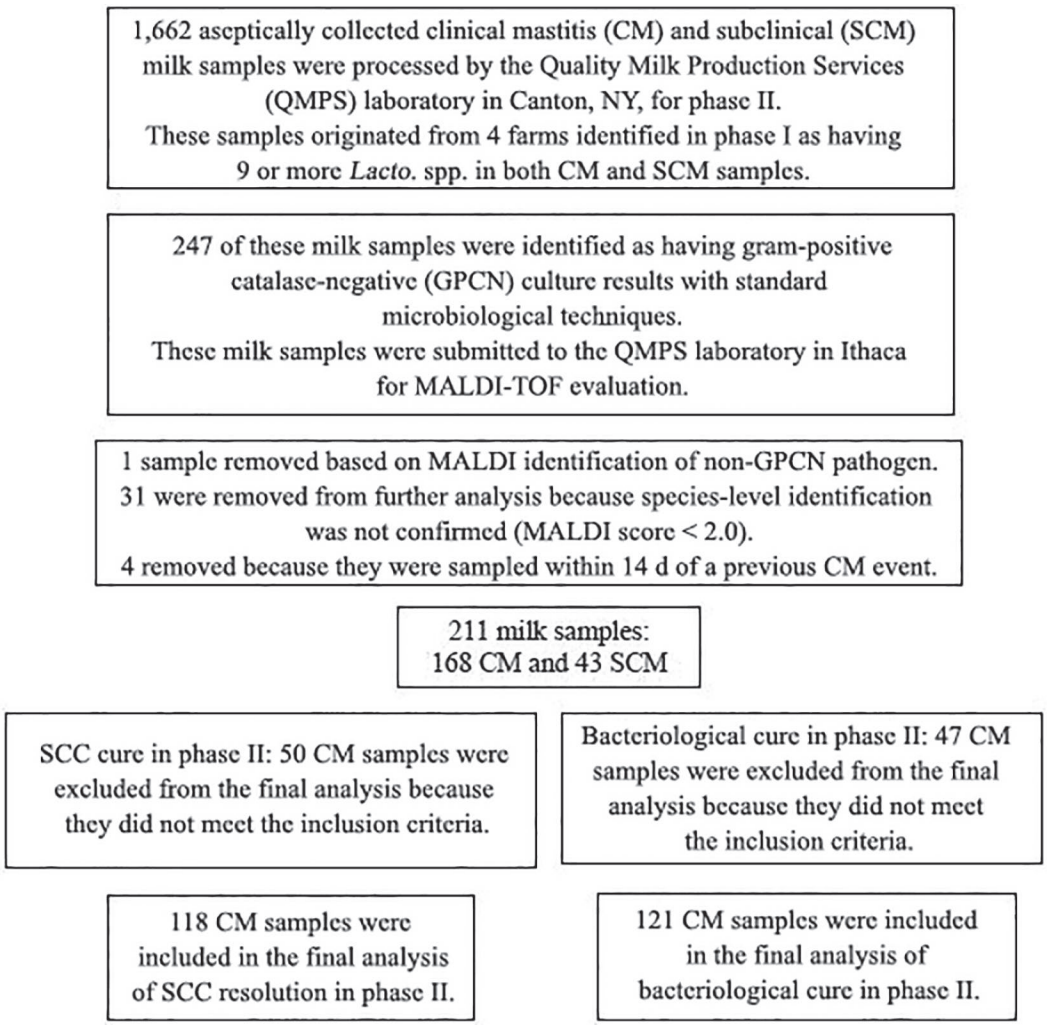

Figure 2. Flow diagram of samples included in the final analysis of SCC resolution and bacteriological cure in phase II (April to October 2015).

parity group 1 and 93 were in group 2, and the DIM at diagnosis included 13 cows in early lactation, 59 in mid lactation, and 49 in late lactation. The final model included only the pathogen group (*S. dysgalactiae* or *S. uberis* vs. *L. lactis* or *L. garvieae*) and farm as a random effect.

RESULTS

Phase I

Distribution of GPCN. The distribution of the GPCN in the 473 samples from CM and SCM milk samples from phase I is found in Table 1. Table 2 describes the distribution of pathogenic GPCN from farms in phase I with 10 or more CM samples. Most (214 of 277) of the CM samples originated from 6 farms with an average herd size of 1,120 (range 500–1,975). From these 6 farms, one pathogen group was cultured in the majority (at least 54%) of the CM samples. Three of the 6 farms had *Lactococcus* spp. as the major pathogen in CM samples, while 2 farms had *S. dysgalactiae* and 1 farm had *S. uberis* as the major pathogenic GPCN.

SCC Resolution (SCC ≤200,000 cells/mL) in Composite Milk Samples. In phase I, which included 127 CM samples available for analysis, 73 samples were found to contain *S. dysgalactiae* or *S. uberis* (68% with SCC resolution) and 54 had *L. lactis* or *L. garvieae* (26% with SCC resolution), with a risk difference of 40%. In these 127 composite milk samples, irrespective of SCC resolution status, the mean linear score was 3.3 for samples with a *S. dysgalactiae* or *S. uberis* culture result and 5.0 for those with *L. lactis* or *L. garvieae*. Cows with CM and *S. dysgalactiae* or *S. uberis* identified in the sample had higher odds (6.1) of a SCC resolution than those with *L. lactis* or *L. garvieae* (Table 3).

Table 1. Distribution of gram-positive, catalase-negative cocci (GPCN) identified by MALDI-TOF in milk samples originating from 92 New York State dairy farms from May to November 2014 during phase I[1] of the study

Organism	CM[2]	SCM[3]	Unknown reason	Total	% of cases (from 473)
Aerococcus viridans	—	2	—	2	0.4
Enterococcus casseliflavus	1	—	—	1	0.2
Enterococcus faecalis	1	1	1	3	0.6
Enterococcus faecium	1	—	—	1	0.2
Enterococcus saccharolyticus	15	7	—	22	4.7
Lactococcus garvieae	6	8	2	16	3.4
Lactococcus lactis	91	18	3	112	23.7
Streptococcus dysgalactiae	113	23	19	155	32.8
Streptococcus gallolyticus	2	4	—	6	1.3
Streptococcus oralis	1	—	—	1	0.2
Streptococcus parauberis	1	1	1	3	0.6
Streptococcus suis	1	—	—	1	0.2
Streptococcus uberis	44	62	44	150	31.7
Total	277	126	70	473	100

[1]During phase I, a convenience sample of 8,868 milk samples (including clinical mastitis, subclinical mastitis, and those sampled for unknown reasons) were evaluated through culture; 495 were identified as having GPCN. Of these, 473 were confirmed by MALDI-TOF with a score of ≥2.0.

[2]Trained on-farm personnel diagnosed clinical mastitis (CM) cases cow-side based on the presence of abnormal milk.

[3]Subclinical mastitis (SCM) cases include those sampled as fresh cows for routine screening (n = 10), those sampled during whole-herd samplings screening for contagious pathogens (n = 101), and those sampled due to high SCC after DHIA testing (n = 15).

Phase II

Distribution of GPCN. Table 4 shows the distribution of GPCN in the 168 CM samples from the 4 farms. Farms A to D are the same farms found in Table 2. Farm size ranged from 500 to 2,300 lactating cows, all of which were housed in freestall barns. Three of the 4 farms had *Lactococcus* spp. as the major pathogen in CM samples and the remaining farm had *S. dysgalactiae* as the major pathogenic GPCN.

SCC Resolution (SCC ≤200,000 cells/mL) in Composite Milk Samples. Among the 118 samples available for analysis, 52 samples contained *S. dysgalactiae* or *S. uberis* (65% with SCC resolution) and 66 had

Table 2. Distribution of gram-positive, catalase-negative cocci (GPCN) from New York State dairy farms with 10 or more positive clinical mastitis[1] samples submitted from May to November 2014 during phase I[2] of the study

	GPCN count of samples (% of total samples by farm ± 95% CI)				
Farm	*Streptococcus dysgalactiae*	*Streptococcus uberis*	*Lactococcus* spp.[3]	Other[4]	Total no. of samples
A	15 (19 ± 9)	7 (9 ± 6)	49 (62 ± 11)[5]	8 (10 ± 7)	79
B	7 (26 ± 17)	1 (4 ± 7)	18 (67 ± 18)[5]	1 (4 ± 7)	27
C	15 (58 ± 19)[5]	1 (4 ± 7)	9 (34 ± 18)	1 (4 ± 7)	26
D	5 (26 ± 19)	1 (5 ± 10)	12 (60 ± 21)[5]	2 (10 ± 13)	20
E	43 (88 ± 9)[5]	4 (8 ± 8)	0 (0)	2 (4 ± 6)	49
F	4 (30 ± 25)	7 (54 ± 27)[5]	0 (0)	2 (15 ± 20)	13
Total	89	21	88	16	214

[1]Trained on-farm personnel diagnosed clinical mastitis (CM) cases cow-side based on the presence of abnormal milk.

[2]During phase I, a convenience sample of 143 farms submitted 8,868 milk samples (including clinical mastitis, subclinical, and those sampled for unknown reasons), which were evaluated through culture; 495 were identified as having GPCN. From these, 473 were confirmed by MALDI-TOF with a score of ≥2.0. The confirmed samples included 277 CM samples, 214 of which originated from these 6 farms.

[3]Includes *Lactococcus lactis* (n = 84) and *Lactococcus garvieae* (n = 4).

[4]Includes *Streptococcus suis* (n = 1), *Streptococcus gallolyticus* (n = 2), *Streptococcus parauberis* (n = 1), *Enterococcus saccharolyticus* (n = 10), *Enterococcus faecium* (n = 1), and *Enterococcus casseliflavus* (n = 1).

[5]The most commonly isolated pathogen by farm.

Table 3. The odds of SCC[1] resolution by pathogen group[2] in clinical mastitis (CM)[3] milk samples (n = 127) from 10 farms[4] during May to November 2014 during phase I[5] of the study

	Analysis of maximum likelihood estimates			Odds ratio (vs. referent)	
Variable	Estimate	SE	*P*-value	Point estimate	95% CI
Intercept	−0.76	0.3	0.02		
Streptococcus spp.	1.8	0.4	<0.001	6.1	2.7–13.9
Lactococcus spp.	—	—	—	Referent	Referent

[1]SCC resolution defined as DHIA test-day composite SCC ≤200,000 taken between 15 and 60 d after CM case diagnosis.

[2]Pathogen groups defined as *Streptococcus dysgalactiae* or *Streptococcus uberis* versus *Lactococcus lactis* or *Lactococcus garvieae*.

[3]Trained on-farm personnel identified CM cases and submitted milk samples to the laboratory.

[4]Farm treated as random effect.

[5]During phase I, a convenience sample of 143 farms submitted 8,868 milk samples (including CM, subclinical, and those sampled for unknown reasons), which were evaluated through culture; 495 were identified as having gram-positive, catalase-negative cocci. From these, 473 were confirmed by MALDI-TOF with a score of ≥2.0. The confirmed samples included 277 CM samples, but only 127 met eligibility criteria for inclusion in the final statistical analysis.

L. lactis or *L. garvieae* (48% with SCC resolution), with a risk difference of 17%. In these 118 composite milk samples, the mean linear score was 5.2 for samples with *S. dysgalactiae* or *S. uberis* culture results and 6.3 for those with *L. lactis* or *L. garvieae*. Cows with CM and *S. dysgalactiae* or *S. uberis* culture result had higher odds (2.4) of a SCC resolution than those with *L. lactis* or *L. garvieae* (Table 5).

Bacteriological Cure in CM Samples. Among the 121 samples available for bacteriological analysis, 57 samples contained *S. dysgalactiae* or *S. uberis* (93% bacteriological cures) and 64 had *L. lactis* or *L. garvieae* (63% with bacteriological cures), with a risk difference of 30%. The odds of a cow experiencing a bacteriological cure was higher (8.0) if *S. dysgalactiae* or *S. uberis* was present versus *L. lactis* or *L. garvieae* (Table 6).

DISCUSSION

We evaluated the presence of *Lactococcus* spp. in both CM and SCM samples from multiple commercial farms in this study, thus helping to define the distribution of this pathogen and its relationship with other pathogenic GPCN in these sample types. Although the sampling scheme for phase I was based on a convenience sample of 143 farms in northern New York and was not balanced for farm size, location, or other potential confounders, these samples were representative

Table 4. The distribution of gram-positive, catalase-negative cocci (GPCN) in clinical mastitis (CM)[1] samples from the 4 farms selected to be part of phase II[2] of the study (samples submitted from April to October 2015)

	GPCN count of samples (% of total samples by farm ± 95% CI)				
Farm[3]	*Streptococcus dysgalactiae*	*Streptococcus uberis*	*Lactococcus* spp.[4]	Other[5]	No. of samples
A	15 (27 ± 12)	2 (4 ± 5)	32 (57 ± 13)[6]	7 (13 ± 9)	56
B	10 (59 ± 23)[6]	0 (0)	7 (41 ± 23)	0 (0)	17
C	13 (24 ± 11)	11 (20 ± 11)	29 (53 ± 13)[6]	2 (3 ± 5)	55
D	14 (35 ± 15)	5 (12.5 ± 10)	20 (50 ± 15)[6]	1 (2.5 ± 5)	40
Total	52	18	88	10	168

[1]CM identified by presence of abnormal milk by trained on-farm personnel. All CM samples were submitted by the farm for evaluation.

[2]Farms with 9 or more *Lactococcus* spp. from either CM or subclinical CM samples in phase I were selected to be part of phase II.

[3]Farms (A–D) in this table are the same farms included in Table 2 and are presented in the same order.

[4]*Lactococcus lactis* (n = 87) and *Lactococcus garvieae* (n = 1).

[5]Includes *Enterococcus saccharolyticus* (n = 6), *Enterococcus faecium* (n = 1), *Enterococcus thailandicus* (n = 1), *Streptococcus equinus* (n = 1), and *Streptococcus mitis* (n = 1).

[6]The most commonly isolated pathogen by farm.

Table 5. The odds of SCC resolution[1] by pathogen group[2] in clinical mastitis (CM)[3] milk samples (n = 118) from 4 farms[4] in phase II[5]

	Analysis of maximum likelihood estimates			Odds ratio (vs. referent)	
Variable	Estimate	SE	*P*-value	Point estimate	95% CI
Intercept	−0.1	0.5	0.9		
Streptococcus spp.	0.9	0.4	0.03	2.4	1.1–5.5
Lactococcus spp.	—	—	—	Referent	Referent

[1]SCC resolution defined as DHIA test-day composite SCC ≤200,000 taken between 15 and 60 d after CM case diagnosis.

[2]Pathogen groups defined as *Streptococcus dysgalactiae* or *Streptococcus uberis* versus *Lactococcus lactis* or *Lactococcus garvieae*.

[3]Trained on-farm personnel identified CM cases and submitted milk samples to the laboratory.

[4]Farm treated as random effect.

[5]Farms with 9 or more *Lactococcus* spp. from either CM or subclinical CM samples in phase I were selected to be part of phase II.

of those received by the Canton QMPS laboratory and the veterinary clinic during the study period. We found that *Lactococcus* spp. could be commonly isolated in both CM and SCM samples, accounting for up to 27% of all GPCN cases, Table 1.

Lactococcus spp. have recently been referred to as emerging pathogens in CM (Rodrigues et al., 2016). Their emergence as pathogenic species could be related to changes in the environment that facilitate their growth and introduction into the udder or to improved biochemical and advanced molecular techniques in milk quality laboratories enabling accurate identification of *Lactococcus* spp. instead of misclassification as *Streptococcus* spp. (Werner et al., 2014). Given that *Streptococcus* spp. are among the most commonly isolated pathogens in CM samples (Hertl et al., 2014; Oliviera and Ruegg, 2014; Vasquez et al., 2017), it may not be surprising that the use of molecular techniques (e.g., MALDI-TOF) to confirm pathogen identify in our study revealed, for example, that *Lactococcus* spp. were present in 35% of CM cases in phase I (Table 1). The odds of SCC resolution were 6.1 (95% CI: 2.7–13.9; Table 3) times higher in phase I, in which a convenience sample of dairy herds was evaluated, and 2.4 (95% CI: 1.1–5.5; Table 5) times higher in the 4 herds selected for having a high prevalence of *Lactococcus* spp. for samples with *S. dysgalactiae* or *S. uberis* compared with *L. lactis* or *L. garvieae*. The odds of bacteriological cure was 8.0 (95% CI: 2.5–25.6; Table 6) higher for samples with *S. dysgalactiae* or *S. uberis* compared with *L. lactis* or *L. garvieae* in phase II. Due to the differences in SCC resolution and bacteriological cure, proper identification of the causal pathogen in infections is important because cases may be managed differently based on the organism.

As reported, the odds of SCC resolution and bacteriological cure were higher for cows with *S. dysgalactiae* or *S. uberis* culture results compared with *L. lactis* or *L. garvieae*, albeit with some limitations. The SCC was based on a composite DHIA milk sample that can be

Table 6. The odds of bacteriological cure[1] by pathogen group[2] in clinical mastitis (CM)[3] milk samples (n = 121) from 4 farms[4] in phase II[5]

	Analysis of maximum likelihood estimates[4]			Odds ratio (vs. referent)	
Variable	Estimate	SE	*P*-value	Point estimate	95% CI
Intercept	−0.6	0.4	0.2		
Streptococcus spp.	2.1	0.6	0.005	8.0	2.5–25.6
Lactococcus spp.	0	—	—	Referent	Referent

[1]Bacteriological resolution defined as no growth or identification of different pathogen from a quarter sample taken 14 to 28 d after CM diagnosis.

[2]Pathogen groups defined as *Streptococcus dysgalactiae* or *Streptococcus uberis* versus *Lactococcus lactis* or *Lactococcus garvieae*.

[3]Trained on-farm personnel identified all CM cases and submitted milk samples to the laboratory.

[4]Farm treated as random effect.

[5]Farms with 9 or more *Lactococcus* spp. from either CM or subclinical CM samples in phase I were selected to be part of phase II.

affected by other quarters. Additionally, initial SCC evaluation was not done for CM samples. However, it is important to note that SCC resolution was defined as SCC ≤200,000 cells/mL in a composite milk sample. It was defined in this manner to avoid confusing it with a cure, which would have required an initial sample. From a management perspective, farms use the composite SCC to make management decisions for specific cows in a herd, and it is still a useful outcome to measure. In both phase I and II, only CM cases that received an approved IMM antibiotic for mastitis treatment were included in the final analysis. However, sample size was not sufficient to compare the effectiveness of treatment choice or duration. Randomized controlled trials evaluating the effectiveness of certain IMM treatments and durations are necessary to accurately answer this question. Lastly, the statistical analysis focused on comparing 2 pathogen groups composed of *L. lactis* and *L. garvieae* in one group and *S. dysgalactiae* and *S. uberis* in the other; the analysis did not evaluate any potential differences between the species within each group. Evaluation at this level was not possible due to the small number of eligible *S. uberis* and *L. garvieae*. Future studies could balance the data set to include a sufficient number of *S. uberis* for a separate analysis given that this species has also been shown to result in persistent infections (Oliver et al., 2004; Milne et al., 2005).

One reason for initiating this project was to seek answers to producer-driven questions about where *Lactococcus* spp. were coming from and why they seemed to be more prevalent in some herds. Due to previously cited limitations of microbiological identification of some GPCN (Wyder et al., 2011; Werner et al., 2014), the identification of *Lactococcus* spp. as mastitis pathogens was unknown to these farms before the start of this study. We included a limited by-farm characterization of GPCN distribution (Tables 2 and 4), and these data are presented to describe the distribution of GPCN within and between farms. These data may also enable forming hypotheses about possible risk factors for the emergence of *Lactococcus* spp. as mastitis pathogens. Interestingly, some farms in our study did have *Lactococcus* spp. as the predominant pathogens, while others had *Streptococcus* spp.

In an outbreak investigation, Rodrigues et al. (2016) evaluated DNA fingerprinting profiles and concluded that multiple sources for the *Lactococcus* spp. were likely in that outbreak; therefore, it was less likely that a contagious component was present. Although Rodrigues et al. (2016) did not find any evidence of such a component, other pathogens previously classified as originating from environmental sources have been shown to have contagious behavior following further investigation (Munoz et al., 2007). Information on multiple herds over time is needed, in addition to longitudinal evaluation of possible farm-level risk factors that may influence predominant CM pathogens within the GPCN category and assessment of possible transmission modes.

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