



## **Northern NY Agricultural Development Program 2015 Project Report**

### **Longitudinal Characterization of Mastitis-Causing Pathogens Previously Identified as “Other Streptococcal Species”**

#### **Project Leader:**

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#### **Collaborating Producers:**

- Adirondack Dairy, 193 Brown Road, Peru, NY 12972
- Deer Run Dairy, 12065 Bishop Street Road, Adams, NY 13605
- Hy-Light Farms, 13723 County Route 75, Adams, NY 13605
- Lloyd T. Smith and Sons, 2717 County Route 27, Canton, NY 13617
- Windsong Dairy, 20977 Fassett Road, Adams Center, NY 13606

#### **Background:**

Mastitis is the most common and costly disease of dairy cattle, with losses close to \$200 per cow per year in the US (Bar, et. al, 2007). Environmental organisms cause the majority of clinical mastitis infections (Hogan, 1989; Makovec and Ruegg, 2003) in herds with low somatic cell count (SCC), including 25.4% of which are environmental Streptococci, specifically Streptococcus species other than Streptococcus agalactiae (GPCN).

When evaluating mastitis on five farms in New York State, the largest percentage of clinical mastitis cases was attributed to *Streptococcus* species, regardless of which case of mastitis the cow was experiencing during the lactation(Hertl, 2014).

GPCN organisms account for a large number of cases of clinical and subclinical mastitis in the dairy industry(Jones, 2009). *Streptococcus uberis* is the most commonly isolated pathogen from cows with clinical mastitis in Flanders at 18% of samples, *Streptococcus dysgalactiae* was identified in 7.2% of samples, and other esculin-positive cocci other than *Strep uberis* were identified in 2.1% of samples(Verbeke, 2014). These environmental GPCN organisms can be associated with chronic mastitis late in lactation (Wyder, 2011), especially *Lactococcus garviae*, with increases in SCC when compared to control animals, indicating that this organism may be a significant mastitis causing pathogen.

Identification of *Streptococcus agalactiae* and *Strep dysgalactiae* using standard microbiological methods are very accurate when compared to 16S sequencing (Wyder, 2011). However, the GPCN category contains a large number of “other Streptococci” organisms, in addition to including the genus *Lactococcus*, *Enterococcus*, and *Aerococcus*, which cannot be easily differentiated using standard biochemical tests(Fortin, 2003).

*Lactococcus lactis* appears to be difficult to differentiate phenotypically from other *Streptococcus* organisms, indicating that this group is likely misidentified and unreported as a potential cause for clinical mastitis(Plumed-Ferrer, 2013; Werner, 2014).

The comparison of biochemical tests used for standard microbiological methods to Matrix Assisted Laser Desorption/Ionization-Time of Flight (**MALDI-TOF**) during the Northern New York Agricultural Development Program (NNYADP) 2014 research project highlighted this issue with current identification methods and led Quality Milk Production Services (**QMPS**) to explore and incorporate new biochemical tests into identification protocols, significantly increasing the accuracy of identification of *Lactococcus* species and reporting this results for clients.

Through a NNYADP research project funded in 2014, Quality Milk Production Services identified Streptococci-like organisms in 473 cows on 83 farms between May and October 2014 and identified:

- *Streptococcus dysgalactiae* (n=155; 32.8%; 35 farms)
- *Streptococcus uberis* (n=150; 31.7%; 76 farms)
- *Lactococcus lactis* (n=112; 23.7%; 19 farms)
- *Lactococcus garviae* (n=16; 3.4%; 11 farms)
- *Enterococcus saccharolyticus* (n=22; 4.7%)
- other *Streptococcus*, *Enterococcus* and *Aerococcus* species (n=18, 3.7%; 13 farms)

**Previous to that NNYADP-funded work, many of these organisms would have been misidentified or categorized into a broad group** where there is little research into characterizing the mastitis caused by the organisms included in the category.

Many of these species historically have not been considered significant pathogens in bovine mastitis and the role of these microorganisms as economically-important agents remains unclear. Important information that this still not known about this group of organisms includes:

- response to therapy
- effect on milk production
- risk of chronic infection
- risk of culling

The objective of this study in 2015 was to better characterize the infections and outcomes of these GPCN infections on five dairy farms in Northern NY, including bacteriological cure, impact on SCC at the time of infection and after treatment, longevity in the herd, milk production and risk of recurrent mastitis.

#### **Methods:**

Five collaborating farms that were identified in 2014 with >20 animals with GPCN pathogens, specifically *Lactococcus lactis*, were enrolled into the study in April of 2015. All milk samples from those five farms normally submitted to QMPS were cultured using standard microbiological methods established by the National Mastitis Council (NMC Handbook, 1999).

Samples were submitted from animals with clinical mastitis (all farms), fresh cow screening (2 farms) and for high SCC after Dairy Herd Improvement Association (DHIA) testing (2 farms). In addition, chronic subclinical cows were identified after each DHIA test day using a current SCC >200, previous SCC >200 and days carried calf <190 as parameters for identification. A randomly selected subset (based on herd size) of subclinical animals were aseptically sampled by the farm or by QMPS. For a six-month period (April-October 2015), samples diagnosed with GPCN organisms from those two sources were sent overnight to Cornell University Animal Health Diagnostic Center in Ithaca, NY.

All confirmed samples were then speciated using MALDI-TOF (Bruker Daltonics, The Woodlands, TX) technology to confirm the bacteria present to the genus and species level. Bacteria for which the MALDI-TOF results with a score of <2 or for which the MALDI-TOF could not reliably confirm identity of were removed from the study.

All samples included in the study were tested for somatic cell count using a DeLaval Cell Counter (DCC, DeLaval Inc., Tumba, Sweden). Results were either numerical or resulted in a “Flow Error.” Numerical results were transformed into Linear Score (LS), using the equation  $\log_2(\text{SCC}/100,000) + 3$ . Samples that resulted in “Flow Error” were

assessed for SCC using the procedure for direct microscope somatic cell count (DMSCC) as outlined by the National Conference on Interstate Milk Shipments, document M-I-05-3 (FDA form 2400G).

All cows enrolled were resampled 14 to 28 days after the initial sampling in order to assess for bacteriological cure and reassess SCC. Cows that were culled, died or were dried off were no resampled.

All farms were enrolled in monthly testing with DHIA (Dairy One; Ithaca, NY) during the study period, although one farm discontinued SCC testing monthly after March of 2015 and only obtained that information every 4 months. Cows identified with GPCN infections were tracked through Dairy Comp 305 (DC305) records after test day for days in milk (DIM) at time of sampling, parity, milk production, SCC, treatment production and duration (if treatment had been attempted), previous and future incidents of mastitis. Data was collected from the test day before sampling through four test days after sampling.

Continuous variables were analyzed using a one-way ANOVA (DIM and LS) and binary responses (bacteriological cure, SCC “cure,” risk of recurrent mastitis, and longevity in the herd) were compared between organisms using two-by-two tables and Pearson’s Chi-squared tests. The impact on milk production was analyzed using multivariable analysis in SAS (Cary, NC).

### **Results:**

Two hundred and sixty nine (269) cows were identified with GPCN infections from April to October 2015 from the five enrolled farms. Six cows were removed from the data set for the following reasons: 1) the sample was taken under 14 days after a previous sample was identified with a GPCN infection (3 cows), 2) the animal was a prefresh heifer (1 cow), 3) more than one organism was present (mixed infection, 1 cow) and 4) a pathogen which was not a GPCN organism was identified with MALDI-TOF (*Histophilus somni*, 1 cow). Twenty-four (24) cows were only able to be accurately identified to the genus level by MALDI-TOF. An additional 12 cows were not included in the analysis because organism was unable to be confirmed with MALDI-TOF (result of “no reliable identification”).

Two hundred and twenty-nine (229) animals were included in the final dataset with accurate identification of the organism present. Of the organisms identified,

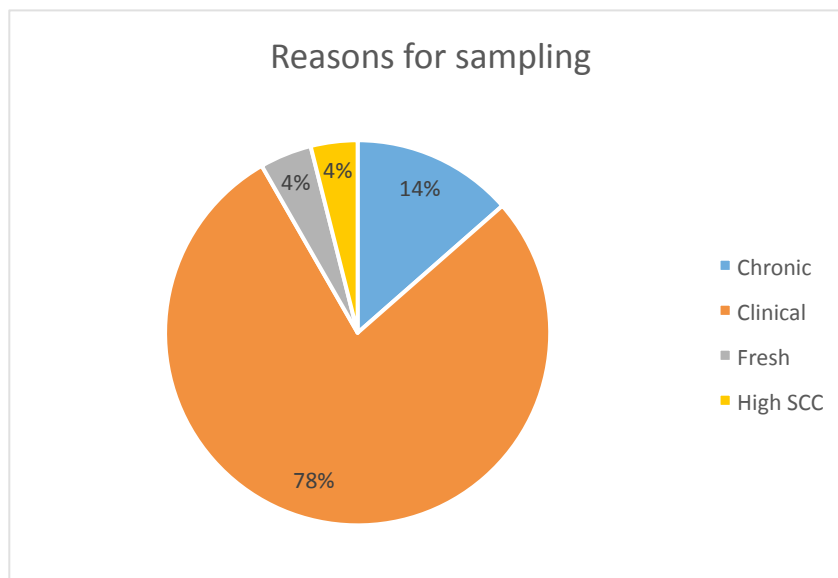
- 67 were *Streptococcus dysgalactiae* (26.5%)
- 28 as *Streptococcus uberis* (11.1%)
- 118 as *Lactococcus lactis* (46.6%)
- 2 as *Lactococcus garviae* (0.8%)
- 6 as *Enterococcus saccharolyticus* (2.4%)
- 3 as *Enterococcus faecium* (1.2%)
- 2 as *Enterococcus faecium* (0.8%)
- 1 each of *Enterococcus thailandicus*, *Streptococcus equinus* and *Streptococcus mitis* (0.4% each; Table 1).

**Table 1. Distribution of GPCN organisms identified from clinical, subclinical, fresh and high SCC cows from 5 farms in Northern New York, April to October 2016.**

<b>Organism</b>	<b>Farm A</b>	<b>Farm B</b>	<b>Farm C</b>	<b>Farm D</b>	<b>Farm E</b>	<b>Totals by organism</b>
<i>Streptococcus dysgalactiae</i>	15	15	16	6	15	<b>67</b>
<i>Streptococcus uberis</i>	2	13	5	7	1	<b>28</b>
<i>Streptococcus equinus</i>	1	0	0	0	0	<b>1</b>
<i>Streptococcus mitis</i>	0	1	0	0	0	<b>1</b>
<i>Lactococcus lactis</i>	31	41	30	4	12	<b>118</b>
<i>Lactococcus garvieae</i>	1	0	0	0	1	<b>2</b>
<i>Enterococcus saccharolyticus</i>	6	0	0	0	0	<b>6</b>
<i>Enterococcus faecium</i>	0	0	2	0	0	<b>2</b>
<i>Enterococcus faecalis</i>	0	0	0	1	2	<b>3</b>
<i>Enterococcus thailandicus</i>	0	1	0	0	0	<b>1</b>
<b>Totals by Farm</b>	<b>56</b>	<b>71</b>	<b>53</b>	<b>18</b>	<b>31</b>	<b>229</b>

Of the 229 animals identified to the species level, 179 were identified from clinical mastitis samples (78.1%), 1 was from fresh cow screenings (0.4%) and 9 were from samples taken of high SCC animals as part of evaluation after test day (3.9%).

During the study period, 2,318 samples were submitted to QMPS for the reasons stated earlier. In addition, 31 animals were included because of samples taken of chronically high SCC randomly picked after test days for screening as part of the study out of 269 chronic cows sampled (12.6% of subclinical cows, 13.5% of dataset, Figure 1).



**Figure 1: Percentage of each type of sample (farm reason for sampling cow) included in dataset.**

### **Days in Milk (DIM) at Sampling**

DIM at the time of sampling was compared for all 179 clinical cases. Chronic, fresh cow and high SCC cases were not included in this comparison as the sampling dates for those cases are based on external decision making factors and other pieces of information that are not consistent. There was no difference ( $p>0.05$ ) in the DIM at the time of clinical signs when comparing organisms:

- *S. dysgalactiae* (161 DIM, n=57)
- *S. uberis* (157 DIM, n=23)
- *L. lactis* (189 DIM, n=88)
- *L. garviae* (208 DIM, n=1)
- *S. mitis* (161 DIM, n=1)
- *S. equinus* (77DIM, n=1)
- *E. saccharolyticus* (174 DIM, n=6)
- *E. faecium* (172 DIM, n=1)
- *E. thailandicus* (2 DIM, n=1)

### **Initial Linear Score**

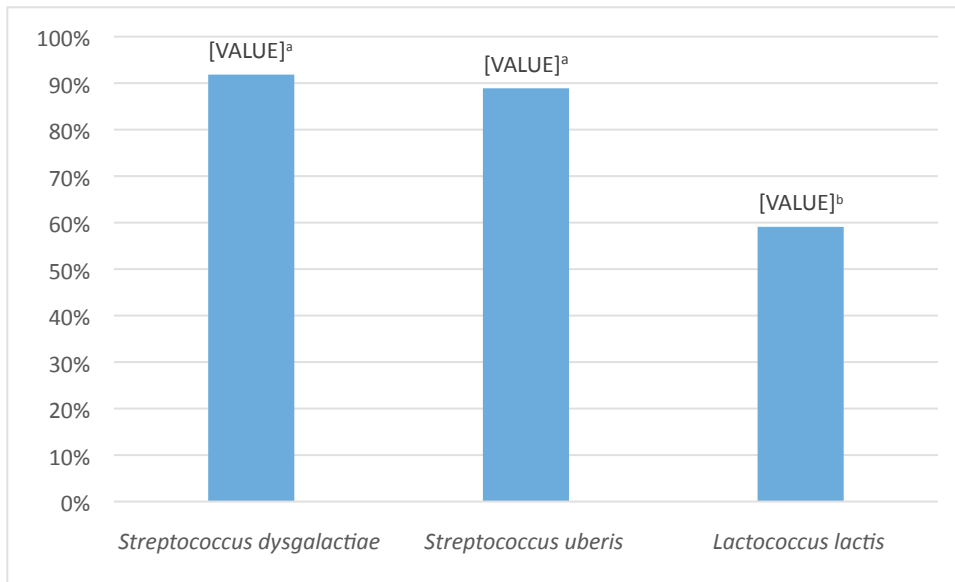
Linear score was compared for only the 179 clinical mastitis samples because other samples were taken at arbitrary time points based on management decisions. There was no difference ( $p=0.08$ ) in linear scores when comparing:

- *S. dysgalactiae* (7.57, n=57)
- *S. uberis* (7.32, n=23)
- *L. lactis* (7.17, n=88)
- *L. garviae* (8.14, n=1)
- *S. mitis* (12.02, n=1)
- *S. equinus* (8.04, n=1)
- *E. saccharolyticus* (5.84, n=6)
- *E. faecium* (8.45, n=1)
- *E. thailandicus* (7.54, n=1)

### **Bacteriological Cure**

Bacteriological cure was assessed for 142 clinical mastitis cows (2 cows with clinical mastitis were not treated and excluded from the comparison) after administration of intramammary antibiotics (any product for any duration). There were not enough samples from chronically infected cows that were not treated to assess spontaneous cure without therapy. Cows were classified with a bacteriological cure if the follow-up sample was negative or if it cultured positive for a different pathogen than originally identified (assumed cured and then infected with a new pathogen). Bacteriological cure for other infections were not compared due to low sample size (n=9).

**There was a significant difference between the bacteriological cure rate for *Lactococcus lactis* (59% cures, n=66) when compared to both *Streptococcus dysgalactiae* (92% cures, n=49,  $p<0.001$ ) and *Streptococcus uberis* (89% cures, n=18,  $p=0.02$ ; Figure 2).**



<sup>ab</sup> Percentage bacteriological cures with different superscripts are significantly different from one another ( $p < 0.05$ ) using a chi square analysis.

**Figure 2. Bacteriological cure rate by comparing milk culture result taken 2-4 weeks after original sample date if animal remained in herd (n=133). Analysis does not take into account treatment product or duration. Pathogens that infected <10 cows not included.**

The most common treatment product used was Today (Boehringer Ingelheim, St. Joseph, MO) and bacteriological cure for cows treated with Today versus all other products was not different for any pathogen ( $p > 0.05$ ).

### **Somatic Cell Count (SCC) Resolution**

SCC resolution was defined as a SCC under 200,000 on the follow-up sample 2 to 4 weeks after initial identification. Only samples from clinical cows that were treated were compared (n=151). When comparing SCC resolution for clinical cows treated with any product(s), 24% of *Strep dysgalactiae* (n=55) and 18% of *Lactococcus lactis* (n=67) were resolved which was not significantly different ( $p > 0.05$ ). All other pathogens were not compared due to small sample size (<10 samples).

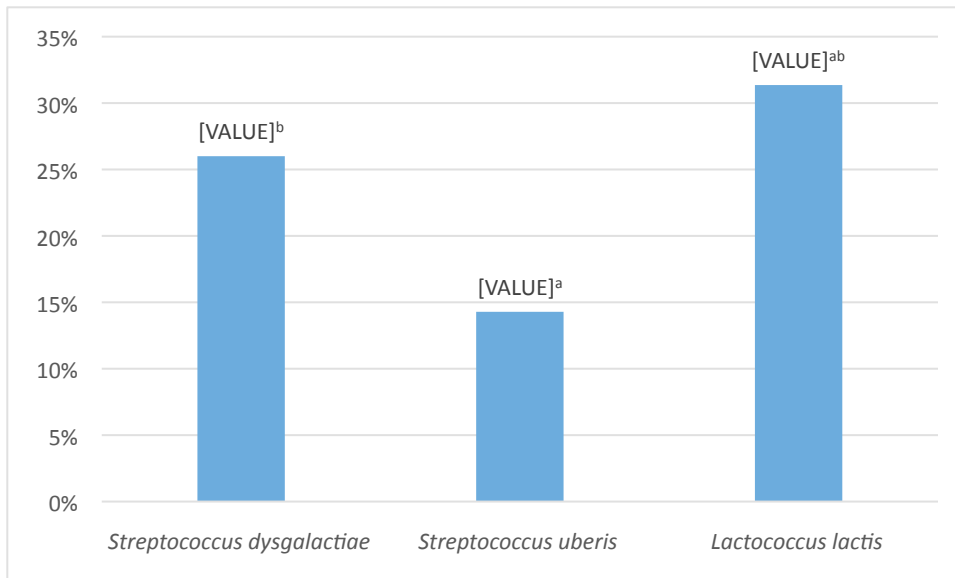
### **Risk of Recurrent Mastitis**

All cows were tracked for four months after sampling through DHIA records to evaluate for recurrent mastitis (n=229). At least a second clinical mastitis event was recorded for:

- 26% of cows with *Streptococcus dysgalactiae* (n=67)
- 14% of *Streptococcus uberis* (n=28)
- 31% of *Lactococcus lactis* (n=118)
- 0% of *Enterococcus saccharolyticus* (n=6)

Other pathogen groups with only 1-2 cows with those infections were not included.

**The difference between *S. uberis* and *L. lactis* indicated a potential trend for an increased risk of recurrent mastitis for those cows with *L. lactis* infections ( $p=0.10$ , RR=2.2; figure 3).**



<sup>ab</sup> Risk of subsequent mastitis events with different superscripts indicate a potential trend ( $p < 0.1$ ) using a chi square analysis

**Figure 3: Percentage of cows with recurrent mastitis after each type of infection. Subsequent mastitis events were tracked for 4 months in dairy comp and includes animals with one or more additional cases of clinical mastitis in that time period.**

### **Risk of Leaving the Herd**

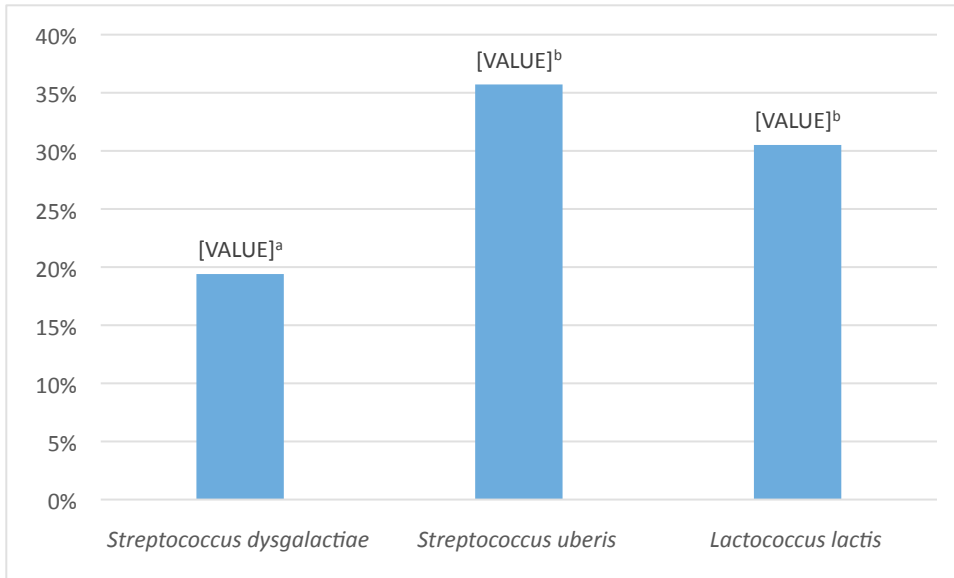
Of those cows with *Streptococcus dysgalactiae* infections, 13 were sold (19%, n=67); of those with *Streptococcus uberis*, 1 died and 9 were sold (36%, n=28); of those with *Lactococcus lactis* infections, 36 were sold (31%, n=118); and of those with *Enterococcus saccharolyticus* infections, one cow was sold (17%, n=6). Cows that were dried off during that time period were classified as staying in the herd. Those animals that were noted to have been culled, sold or died were classified as having left the herd.

**There was a trend towards a difference in number of cows leaving the herd after a *Streptococcus dysgalactiae* infection (19%) compared to those with *Streptococcus uberis* (36%,  $p=0.09$ ) or *Lactococcus lactis* (31%,  $p=0.10$ , figure 4).**

### **Effect on Milk Production**

Impact of infection on production was evaluated by comparing test day milk production from the test before sampling as long as the test day SCC was under 200,000 cells/ml (in order to screen for those already subclinically infected) to milk production recorded up to 28 days after sampling. The data was modeled using SAS, taking also parity, DIM, pathogen and bacteriological cure into account. **The only parameter found to affect milk production was DIM ( $p < 0.0001$ ).** Species of bacteria causing the intramammary infection (IMI) did not appear to cause different impacts on milk production.





<sup>ab</sup> Risk of leaving the herd with different superscripts indicate a potential trend ( $p < 0.1$ ) using a chi square analysis

**Figure 4: Percentage of cows that leave the herd (culled or died) within four months of each type of infection.**

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### **Conclusions/Outcomes/Impacts:**

*Lactococcus* species mastitis appears to continue to emerge as a pathogen of concern for some dairy farms.

Due to research conducted through the Northern New York Agricultural Development Program, QMPS has begun to identify *Lactococcus* species routinely by refining microbiological procedures and continues to identify this pathogen on farms in NY State regularly.

Research conducted in 2015 supports findings from the study conducted in 2014, further supporting the conclusion that mastitis caused by *Lactococcus lactis* is less likely to result in a bacteriological cure after treatment and potentially have an increased risk of a second case of clinical mastitis and leaving the herd.

### **Outreach:**

Results of this 2015 research project were presented at the 12<sup>th</sup> annual Northern New York Dairy Institute: February 16 and 17, 2016, and the NNYADP Annual ENNY Meeting, Chazy, February 5, 2016, and mentioned in a brief announcement at the NNYADP Annual WNNY Meeting, Watertown: February 12, 2016

Results will be submitted for presentation at the American Association of Bovine Practitioners, Research Summaries, September 2016, and the National Mastitis Council, Technology Transfer Session, February 2017.

### **Next Steps:**

*Lactococcus* species have previously been grouped with other gram positive, catalase negative organisms (*Streptococcus* spp.) in bulk tank milk, bedding and other environmental cultures, and our knowledge of their presence in these samples is limited. Currently, *Lactococcus* species are presumed to act as environmental pathogens and would be managed accordingly. However, because *Lactococcus* has not been previously considered a major mastitis pathogen, its occurrence in bedding and other environmental sources (which can put cows at risk for IMIs) has not been investigated. Additionally, because *Lactococcus lactis* has been identified as a major mastitis pathogen, putting cows at risk for both chronically high somatic cell counts and culling on some NNY farms, its role in IMIs needs further investigation. We propose future research to further our knowledge in this critical area in support of the NNY dairy industry.

### **Acknowledgments:**

This project would not have been possible without the assistance of the five collaborating dairy farmers in Northern NY who submitted samples to QMPS and allowed us to resample their cows for follow-up microbiology. A special thanks goes to the staff of Quality Milk Production Services in Canton, NY, along with our summer intern Mary Bregg, LVT.

### **Reports and/or articles**

- *Lactococcus* and “other *Streptococcus* species” mastitis. Certified Milk Inspector Newsletter. September, 2015.
- *Lactococcus* mastitis – it’s out there. Hoard’s Dairyman. August 25, 2015.
- A peer-reviewed manuscript will be sent to the Journal of Dairy Science.

### **For More Information:**

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