**NE-1748 Multi State Research Project** **Annual Meeting**

**Mastitis Resistance to Enhance Dairy Food Safety**

Annual Business Meeting

Chicago, IL

November 2, 2017

1. The 2017 (FY17) annual business meeting of the NE 1748 Multistate research project was called to order at 5:00 pm by Kasey Moyes.
2. Introduction of members and new members:

Members present: 10 stations represented

Bill Owens Louisiana Louisiana State University

Kasey Moyes Maryland University of Maryland

Ron Erskine Michigan Michigan State University

Sandra Godden Minnesota University of Minnesota

John Middleton Missouri University of Missouri

Pamela Adkins Missouri University of Missouri

Sheila Andrew Connecticut University of Connecticut

John Barlow Vermont University of Vermont

Massimo Bionaz Oregon Oregon State University

Odessa Kerrodeao Tennessee University of Tennessee

Amanda Stone Mississippi Mississippi State University

1. No new members were added. There was brainstorming as to who else might be invited to join the group and the Chair agreed to contact those people.
2. There was no Project Administrator’s report.
3. Pamela Adkins was nominated to serve as NE1748 secretary, agreed to accept the nomination, and was elected.
4. The 2018 meeting to be (tentatively) held October 24 to 26 in Chicago. Room rates will be $199 per night.
5. Seeing no other business the meeting was adjourned after a motion and second.

# NE-1748 ANNUAL REPORT WORKSHEET (FY17)

**OBJECTIVE 1:** Characterize host mechanisms and pathogenic virulence factors associated with mastitis susceptibility and resistance to improve economic outcomes and animal welfare.

Utah State University

Member: David Wilson

Contributors: Kerry Rood, Justine Britten, Zhongde Wang, Jacqueline LaRose Kurz, E Jane Kelly

Genome-wide association study (GWAS) comparing bovine nucleotide differences (SNPs) between cows repeatedly mastitic vs. cows continually free of mastitis was completed. More than 100 candidate SNPs and > 25 quantitative trait loci (QTLs) associated with resistance to mastitis, both clinical and subclinical, were identified. Ten QTLs identified have not been reported previously. Known associations of the QTLs were with teat and udder characteristics and SCC. Bovine mammary epithelial cells (MECs) from mastitis-resistant and mastitis-susceptible cattle differed in expression of genes relevant to inflammation following LPS challenge. Gene expression for 3 chemokines was significantly upregulated after challenge in resistant cows’ MEC, while 40 genes were downregulated. Susceptible cows’ MEC upregulated one gene, and 83 others were not significantly different in regulation following challenge.

Michigan State University

Members: Ronald Erskine, Lorraine Sordillo, Andres Contreras

Contributors: Phil Durst, Ruben Martinez, Stan Moore

We continued to study the impact of negative energy balance and fat mobilization vascular endothelial and adipose inflammatory responses of in vitro by changing the expression of important inflammatory mediators. In particular, the role of n-3 fatty acid content in altering the profile of vasoactive eicosanoids and the role of poly-unsaturated fatty acids on attenuating endothelial cell inflammatory responses, as well as lymphocyte expression of pro-inflammatory cytokines was a central aim of our work.

Hill Farm Research Station, LSU AgCenter

Member: William E. Owens

Mastitis pathogens were identified from milk and goat samples submitted to this laboratory. Organisms were evaluated for antimicrobial susceptibility to a battery of antibiotics to determine therapeutic efficacy.

University of Maryland

Member: Kasey Moyes

Collaborators: R.A. Erdman,T. Elsasser. M.A. Crookenden, J.R. Roche, K.L. Ingvartsen, T. Larsen, M. Garcia, D. Conner, W. Rhoads.

Researchers at the University of Maryland continue to study the effect of nutrient supply (mainly amino acids and glucose) on the immune response (primarily neutrophils and macrophages) during mastitis. Scientists are currently collaborating with those in New Zealand (Crookenden and Roche) examining the relationship between body condition and neutrophil function as well as researchers in Denmark (Ingvartsen and Larsen) with similar objectives regarding nutrition and immune response. Scientists at the University of Maryland are currently collaborating with industry professionals (Conner and Rhoads) regarding the response of macrophages and mammary epithelial cells to various levels of chromium and how this affects the immune response. In collaboration with others at the University of Maryland (Erdman), we have identified an alternative milk collection technique that is noninvasive to the mammary gland during mastitis. Maryland continues to collaborate with scientists at the USDA, Beltsville (Elsasser), to identify the distribution of vitamin E isoforms in various tissues as well as immune cells (neutrophils), blood and milk and their use as antioxidants.

Oregon State University

Member: Massimo Bionaz

Collaborators: Charles Estill, Shelby Filley, Erminio Trevisi (Universita Cattolica del Sacro Cuore, Italy), Johan Osorio (South Dakota State University), Fernanda Rosa, Shana Jaff, Pedram Rezamand (University of Idaho)

We repeated the experiment previously published (Rosa et al., 2017, see publications) by treating dairy goats with a putative PPARγ activator (2,4-thiazolidinedione or TZD) and inducing subclinical mastitis using Strep. uberis. In this experiment and different that the prior one, goats were in optimal body condition and a NRC balanced diet was provided. We have evaluated also the transcriptome of macrophages isolated from milk and liver which were not evaluated in the original published experiment. Even though the goats treated with TZD presented a better response to sub-clinical mastitis compared to control goats, we were unable to replicate the original findings when we used goats in less-then-optimal condition without balancing the diet, especially for vitamin A. We determined that goats in the present experiment had a higher level of vitamin A in blood. Data also indicated that TZD is a weak activator of PPARγ and tend to activate other PPAR isotypes. Therefore, we concluded that TZD had a minor effect on the immune response when animals are in good condition.

Member: Massimo Bionaz

Collaborators: Fernanda Rosa, Wancheng Sun (Qinghai university,China)

We determined that the cytokeratin 8 is not a good marker for isolating mammary epithelial cells from the milk of goats and cows and we proposed a new markers. Validation of the marker is ongoing.

University of Tennessee

Member: Gina Pighetti

Contributors: Reta Duguma Abdi, Desta Beyene Enserum, Susan Headrick, Leszek Wojakiewicz, Caitlin Merill (MS), Jacqueline Vaughn (MS), Tori Couture (MS)

Regions in the bovine genome have been identified that are associated with phenotypes reflecting the strength of immune response to Streptococcus uberis infection and S. uberis concentrations in milk following intramammary challenge. These regions have provided several novel candidate genes that are being examined for their role in mastitis resistance and their potential as novel targets for mastitis control. This research was a value-added experiment to the project funded through USDA-NIFA-AFRI 2011-67015-30168. Experiments conducted in vitro also support the hypothesis that growth of S. uberis in milk from post-partum dairy cows concentrations varies with genetic background.

State University of New Jersey

Member: Jeff Boyd

Host abscesses are often devoid of oxygen. This is often the result of neutrophil recruitment and activity. We have determined that during anaerobic growth, the bacterial pathogen Staphylococcus aureus alters results in alters the production of S. aureus toxins and increases biofilm formation. Iron restriction also causes S. aureus to ferment. Lactoferrin prevents S. aureus for acquiring iron in the udder. Therefore, host environments that promote fermentative growth, such as the udder, could be causing S. aureus to alter the expression of it’s virulence repertoire.

Washington State University

Member: Larry Fox

Neutrophil functions as affected by dexamethasone and four opsonized M. bovis strains or dexamethasone and four unopsonized M. bovis strains respectively. Dexamethasone consistently decreased all neutrophil functions tested with opsonized or unopsonized M. bovis. There was strain by treatment interaction effect on some neutrophil functions. This interaction had an additive effect on neutrophil killing. The additive or interactive effects of M. bovis and dexamethasone on bovine neutrophil function might explain in part the association of stressful events with subsequent outbreaks of Mycoplasma bovis associated diseases. The stress model may aid in further study of the interaction between M. bovis and stress for evaluating new control measures for M. bovis infection in cattle.

University of Vermont

Members: David Kerr

We are using dermal fibroblast cultures to model cow-to-cow variation in mastitis severity following experimental challenge. Some relation is apparent between the in vitro responses to stimulation with bacterial components and the in vivo responses to experimental challenge with *E. coli*. However, it is proving very difficult to define genetic parameters contributing to the wide variation in how individual animals respond to a defined experimental challenge with viable bacteria.

University of Tennessee

Member: Gina Pighetti

Contributors: Reta Duguma Abdi, Desta Beyene Enserum, Susan Headrick, Leszek Wojakiewicz, Caitlin Merill (MS), Jacqueline Vaughn (MS), Tori Couture (MS)

S. aureus tends to cause chronic mastitis and treatment with antibiotics has limited success. Although several vaccines are available, none confer protection. TN has evaluated the immune response and protection induced in Holstein dairy cows vaccinated with Staphylococcus aureus surface proteins (SASP) or Staphylococcus chromogenes surface proteins (SCSP). Three doses of vaccine were given subcutaneously at 88, 74, and 60 d prior to expected calving. This regimen was capable of increasing specific milk and serum antibody titers. Bacterial shedding in milk also was reduced following a dip-based challenge with S. aureus strain UT1. Interestingly, SCSP vaccine induced cross-protection against S. aureus clinical mastitis whereas SASP induced partial protection.

Staphylococcus aureus is an important zoonotic pathogen which has significant impact on animal and human health. Identifying specific genetic patterns and ability to produce enterotoxins BCD) can provide potential insight for vaccine development. Research to date has indicated that approximately 32% of 118 isolates produced enterotoxins with seB being the most prevalent (18.6%). Two –to three dominant clones were identified.

State University of New Jersey

Member: Jeff Boyd

Biofilm formation is thought to be prerequisite for Staphylococcus aureus infections. Bacteria within biofilms can cause chronic and reoccurring infections. We have discovered that S. aureus responds to fermentative growth by increasing biofilm formation. The biofilm formation phenotype is the result of increased expression of fibronectin binding protein and cell lysis. Cell lysis resulted in DNA release, which contributed to intracellular adhesion. We identified two regulatory systems (SaeSR and SrrAB) that respond to decreased cellular respiration. Both regulatory systems are required for S. aureus pathogenesis. SaeSR is required for exotoxin production. Therefore, environments that promote fermentative metabolism increase the expression of virulence factors via altered SaeSR and SrrAB output.

University of Maine

Member: Anne LIchtenwalner

In vitro trials of alternative treatments, including preliminary evaluation of aromatic compounds and the effects of bedding, for prototheca mastitis in cattle continued.

**OBJECTIVE 2:** Assess and apply new technologies that advance mastitis control, milk quality and/or dairy food safety.

University of Montreal

Members: Jean-Philippe Roy, Mario Jacques, Denis Haine, David Francoz

Researchers at the University of Montreal completed a study on use of an extended pirlimycin treatment for early lactation treatment of *Staphylococcus aureus* in first lactation cows. Firstly, they observed a relatively high spontaneous cure rate (34.1%) for first lactation cows infected with *S.aureus* early during the lactation (first week in milk). The extended pirlimycin treatment yielded significantly higher cure rate (64.8%) compared to no treatment.

Researchers at the University of Montreal demonstrated that matrix assisted laser desorption ionization time of flight mass spectrometry MALDI (MALDI-ToF) can be used to identify a bacterial species in samples prepared from a biofilm. The presence of the biofilm matrix does not seem, with most of the bacterial species tested, to interfere with the analysis when the extended direct transfer method is used.

Using a Bayesian Monte Carlo simulation, researchers at the University of Montreal were able to demonstrate that the selection and misclassification biases resulting from the imperfect accuracy of milk bacteriological culture when used in longitudinal study designs would be very small. Thus, longitudinal studies to estimate *S. aureus* incidence or to report measure of association between an exposure and *S. aureus* incidence could be performed using milk culture of one single sample at recruitment and one single sample at follow-up. The same would apply to other relatively uncommon pathogens (i.e. prevalence < 5%) for which milk culture has closed to perfect specificity (e.g. *Mycoplasma bovis, Streptococcus agalactiae, S. uberis, S, dysgalactiae*). However, for non-*aureus* staphylococci, a more prevalent pathogen (prevalence of 20-40%), these biases can be quite large and cannot be appropriately resolved using sampling strategies making use of duplicate and even triplicate samples at initial and/or follow-up sampling.

Using a systematic literature review, researchers at the University of Montreal confirmed that no evidence-based recommendations could be given for the use of an alternative or a non-antimicrobial conventional treatment for clinical mastitis. Moreover, they demonstrated that probiotics and oxytocin with or without frequent milk out should be avoided. They also concluded that homeopathic treatments are clearly inefficient for management of clinical mastitis.

Cornell University

Members: Daryl Nydam, Loren W. Tauer, Frank Welcome, Yrjo Grohn

Contributors: Yrjo T. Grohn, Loren W. Tauer

Scientists at Cornell University adapted an agent-based whole herd Dairy systems model to study the genetic, technical and financial performance of typical U. S. Dairy herds aiming to lower bulk tank SCC using 6 different practical genomic and phenotypic culling strategies. We are also developing a systems dynamic model which can aid in optimizing antimicrobial use in treatment and prevention of mastitis in typical U.S. Dairy Farms.

University of Minnesota

Members: S. Godden, E. Royster, B. Crooker, R. Fink, T. Schoenfuss, B. Heins, S. Sreevatsan

Contributors: L. Fox (Washington University); P. Ruegg (University of Wisconsin–Madison)

S.M. Godden, E. Royster, J. Timmerman, P. Rapnicki and H. Green carried out an evaluation of an Automated Milk Leukocyte Differential (MLD) Test and the California Mastitis Test for Detecting Intramammary Infection in Early Lactation (EL) and Late Lactation (LL) Quarters and Cows. The objectives of this study were to 1) Describe the diagnostic test characteristics of an automated MLD test (QScout™ MLD. AAD. Durham, NC) and the CMT to identify IMI in EL and LL quarters and cows when using 3 different approaches to define IMI status from milk culture, and 2) Describe the repeatability of MLD test results at both the quarter and cow level. Field sampling and laboratory analysis of samples were completed in spring, 2016. Results showed that diagnostic test characteristics were relatively comparable between the MLD and CMT tests when applied to EL and LL quarters and cows. When applied to EL quarters and cows, both the automated MLD test and the CMT test had only low-to-fair Se, good-to-very good Sp, and only fair-to-good overall Ac, depending on the setting or cutpoint used for interpreting test results. When applied to LL quarter and cows, both tests had fair-to-good Se, fair-to-very good Sp, and fair overall Ac, depending on the setting or cutpoint used for interpreting results. Overall these results suggest that either test may have greater utility in late lactation. However, large randomized field studies evaluating adoption of the MLD or CMT test in SDCT and fresh cow screening programs are needed to describe impacts on udder health, antibiotic use and economics.

Godden, S., T. Schoenfus, R. Fink, J. Timmerman, C. Gebhart, E. Royster, S. Wells performed a feld validation of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-ToF) for the identification of dairy microorganisms critical for safety and quality. The first objective is to develop and evaluate extraction methods to improve the MALDI-ToF method's ability to detect Mycoplasma bovis as well as individual species of Bacillus and Paenibacillus of importance to udder health, food safety and food quality. The second objective is to complete a field validation study using 2,500 bacterial isolates Report Date 02/17/2016 Page 1 of 3 Accession No. 233101 Project No. MIN-62-021 Multistate No. NE1048 derived from bovine mastitis samples and processed dairy foods. From this we will describe the diagnostic test characteristics of MALDI-ToF, as compared to 16S rDNA sequencing (gold standard) to identify 24 important dairy microorganisms critical to animal health, food safety and food quality. If the MALDI-ToF method proves to be accurate, then its adoption will strengthen the capacity, quality, and possibly the scope of diagnostic services performed by the VDL to support the MN dairy industry. Lab work has been completed and a MS student is currently completing data analysis.

E. Royster, Godden, S., J. Timmerman performed a validation of a Rapid On-Farm Culture System for Application in Selective Dry Cow Therapy Programs. The objective is to evaluate the diagnostic test characteristics of a rapid on-farm culture system to identify intramammary infection in individual quarters at dry off, for the purpose of applying selective dry cow therapy. Lab work was completed in summer/fall 2015.

Godden, S., E. Royster, J. Timmerman, B. Crooker, N. McDonald are carrying out a pilot study where they want to evaluate a selective dry cow therapy on udder health. The objective is to complete a pilot study to evaluate the effect of applying a culture-guided selective dry cow therapy program, at the quarter level, on measures of quarter health and antibiotic use. Preliminary data generated from this pilot study was included in a USDA-AFRI grant that we submitted in summer 2017, aiming to complete a much larger multi-state, multi-herd randomized noninferiority clinical trial to evaluate the effect of applying a SDCT program, at the quarter level, on measures of quarter and cow health, lactation performance and economics. Results showed that a culture-guided SDCT program targeting quarter level treatment decisions resulted in equal udder health (vs BDCT) after calving while reducing antibiotic use by 48%.

Patel, K., S. Godden, E. Royster, J. Timmerman, B. Crooker, and L. Fox are investigating the relationship between bacteria counts, bedding characteristics and bedding management practices with udder health and milk quality on dairy farms. The primary objective of this study is to conduct a multi-state, multi-herd cross-sectional observational study to describe the relationship between bedding bacteria counts and udder health and to identify goals (cutpoints) for interpreting BBC test results. A secondary objective is to identify bedding characteristics and bedding management strategies that are associated with lower BBC and improved udder health. One hundred-eighty eight herds were enrolled from 17 dairy states with the assistance of herd veterinarians or mastitis researchers. Herds used either new sand, reclaimed sand, manure solids or other organic bedding materials. New and used bedding samples, collected from the bedding storage area or from the back of stalls, respectively, and bulk tank milk samples were collected twice from each herd during summer and winter of 2016. Bedding samples were cultured to describe TBC, counts of coliform bacteria, non-coliform bacteria, Klebsiella spp., Bacillus spp., Streptococcus spp., and Staphylococcus spp. per cc of bedding material, as well as analyzed to measure pH, organic matter and dry matter. Herd level DHIA test day data describing udder health measures were obtained from the DHIA record processing centers.

S. Godden, E. Royster, B. Crooker and S. Rowe are investigating the relationship between bedding characteristics and intramammary infection in late lactation dairy cows. The major objective of this study is to describe the relationship between bedding characteristics, bedding management and bedding bacteria counts (BBC) in different bedding materials. A secondary objective is to describe the relationship between bedding characteristics and herd-level measures of udder health for late lactation quarters and cows for specific pathogen groups of interest (e.g. Gram-negative organisms; Gram-positive organisms). Additionally, if a relationship is found to exist between BBC and udder health measures, we aim to investigate if specific cutpoints in BBC can be identified as goals for interpreting bedding culture reports. Eighty herds have been enrolled from more than 10 dairy states using one of four bedding types; new sand, reclaimed sand, manure solids or other organic materials. In summer, 2017, sampling of each herd included collection of duplicate aseptic quarter milk samples from late lactation cows (> 180 DCC) as well as new and used bedding samples. Milk and bedding samples are being analyzed by bacterial culture. Additionally bedding samples will undergo testing to measure pH, organic matter and dry matter. Herd sampling will be repeated during the winter of 2017/2018.

University of Missouri

Members: John Middleton, Pamela Adkins

Contributors: Veronique Bernier Gosselin

Our group, in collaboration with University of Calgary and University of Prince Edward Island, has focused on the species and subspecies (strain within species) characterization of staphylococcal bacteria associated with intramammary infection in dairy cattle and dairy goats. We have validated the use of matrix assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry to identify staphylococcal species isolated from both goats and dairy cattle.

University of Maryland

Member: Kasey Moyes

In collaboration with other scientists at the University of Maryland, we have estimated and quantified the production and economic outcomes and lifestyle changes for small–to medium sized dairy farms regarding the transition from conventional to automatic milking systems in the Northeast region. Four farms were enrolled in this study and data has been collected regarding production and economic outcomes relative to their transition. A survey of the enrolled producers was accomplished both before and after their transition to robotic milking regarding their lifestyle changes. Preliminary data will be presented at the National Mastitis Council annual meetings.

Utah State University

Member: David Wilson

Contributors: Kerry Rood, Justine Britten, Zhongde Wang, Jacqueline LaRose Kurz, E Jane Kelly

Blind comparison of bovine milk bacterial identification results between conventional culture, Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and 16S rRNA partial genomic sequencing was completed. Overall agreement between all 3 methods was 87%, with 94% - 100% agreement for most individual pathogens. (Collaboration with Missouri station.)

Data collection has just been completed for study of casein hydrolysate (CH) intramammary infusion for cessation of lactation in all 4 quarters at time of dry-off at the end of lactation. Udder halves were compared between control (dry cow antibiotic treatment plus teat sealant) with 4 treatment groups, each including CH, one being CH alone. Preliminary results include: all quarters of all cows showed no signs of clinical mastitis or cow discomfort and all returned to milk production following calving. All udder halves were not significantly different from 50% of total-cow milk production regardless of treatment group. CH is a non-antibiotic generally regarded as safe substance and the dosage used was 1/500 of that in one feeding of hypoallergenic baby formula. Measures of involution and other outcomes such as SCC and bacteriological results will be analyzed.

Michigan State University

Members: Ronald Erskine, Lorraine Sordillo, Andres Contreras

Contributors: Phil Durst, Ruben Martinez, Stan Moore

As part of a multistate USDA-NIFA funded project, we developed an on-farm evaluation system for milk quality and reduction of antibiotic use. The evaluation system (Quality Milk Alliance) assesses traditional practices related to mastitis control as well as a novel integration with social and communication barriers on the part of dairy producers and employees. We are developing novel applications for VaDia vacuum analysis as a tool to determine milking efficiency in dairy herds. These standards will be used to evaluate milking protocols and equipment performance and have the potential to provide an on-farm education platform for producers and/or employees regarding milking protocols.

Hill Farm Research Station, LSU AgCenter

Member: William E. Owens

Novel food grade products were evaluated for antimicrobial activity. Tea tree oil was shown to be highly effective against mastitis pathogens using the AOAC in vitro test for germicidal activity. The product was also effective against mastitis pathogens in the MIC/MBC test.

University of Tennessee

Member: Gina Pighetti

Contributors: Reta Duguma Abdi, Desta Beyene Enserum, Susan Headrick, Leszek Wojakiewicz, Caitlin Merill (MS), Jacqueline Vaughn (MS), Tori Couture (MS)

The objective of this study was to evaluate the prevalence of antimicrobial resistance and genetic diversity of S. aureus isolates from mastitis cases. Staphylococcus aureus isolates (n = 239) from 33 dairy cattle farms in Tennessee were tested against 10 antimicrobials by broth microdilution method using the Sensititer system. Overall, antimicrobial resistance of S. aureus isolates varied from as low as 1.3% for ceftiofur to as high as 25% for sulphadimethoxine. Out of 239 S. aureus isolates, 82 (34.3%) were resistant to at least one of the 10 antimicrobials tested. The AMR isolates belonged to two major PFGE types indicating the presence of dominant clonal patterns among the resistant isolates. Prevalence of AMR within and among farms varied over time, with an increasing trend in tetracycline resistance.

Washington State University

Member: Larry Fox

A study to determine if mycoplasma mastitis pathogen enumerations differed when incubated under 10% CO2, 5% CO2, or in candle jars (2.7 ± 0.2% CO2) for varying incubation lengths (3, 5, or 7 d) was made. Enumerations of laboratory isolates representing the 3 common mycoplasma mastitis species, *M. bovis*, *M. californicum*, and *M. bovigenitalium*, were not significantly different among CO2 treatments, but were greater after 7 d of incubation compared to 3 d of incubation (all *P* < 0.05). The results were validated against field isolates derived from clinical mycoplasma mastitis bulk tank milk and quarter milk samples. The percentage of field isolates detected was not significantly affected by CO2 treatment. On average, 57.14% of all field isolates were detected by 3 d of incubation compared to 92.86% on d 7. For dairy producers and diagnostic laboratories, allowing 7 d for incubation increases the sensitivity of the test resulting in fewer false negatives and missed cows with mycoplasma infections.

University of Maine

Member: Anne LIchtenwalner

Support was requested to expand quality assessment and control for producers of raw milk (not funded).

University of Vermont

Members: John Barlow

The objective of this study was to explore strain diversity of *Staphylococcus chromogenes* isolates obtained from different dairy farm sources and geographic regions. In collaboration with researchers from Washington and Belgium, 241 S. chromogenes isolates were analyzed using a multilocus sequence-typing (MLST) scheme. Isolates were from USA (n=169) and Belgium (n=48) from various sources. Isolates were also screened for carriage of mecA and blaZ genes. Only five STs (ST1, ST6, ST18, ST29, and ST30) were shared between the two countries. Most STs clustered in four clonal complexes (CC) i.e CC1 (n=12), CC6 (n=9), C15 (n=5), and CC62 (n=7). STs were associated with source, skin versus milk, and geographic region. No isolate was *mecA* positive whereas, *blaZ* gene was present in 21.6% of the isolates and was associated with STs from cow skin.

The objective of this study was to explore the use of machine learning tools for predicting pathogen strain type using metadata available from MLST isolate databases. Complete MLST isolate datasets (PubMLST) for three mastitis pathogens were evaluated using two distinct machine learning models in order to predict strain (sequence) type (ST). Data for a total of 3,912, 1,282, and 988 isolates were used for *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. Models were created using Linear Discriminant Analysis (LDA) and Random Forest (RF) in R. Models were trained on a randomly selected subset of isolates (75%) and accuracy of these predictions tested on the remainder (25%). Important features in the datasets that predicted ST included geography and species specific features (e.g. year of isolation and farm). This work sheds light on potential areas of improvement in database curation and future application for MLST isolate epidemiology.

**OBJECTIVE 3:** Identify and apply new strategies associated with the control of mastitis that can reduce the use of antibiotics in dairy herds.

This objective is new and data has not been collected and/or presented/published for several studies. Scientists at the University of Connecticut (CT) will the use of ultrasound-guided intramammary scanning will be investigated as a tool to provide additional information about IMI and tissue damage that can potentially be used to target dry-off treatment as part of a total program to reduce the amount of antibiotic use in dairy cattle.

The NE-1048 research group at the University of Maine (ME) will assess the impact of paper mill lignin byproducts (PMLBs) as conditioners for dairy bedding, in an effort to help develop products to reduce the incidence of mastitis without additional use of antibiotics. Maine will evaluate selected PMLBs against major mastitis pathogens (bacterial and fungal) *in vitro*, assess their efficacy in the context of commonly used organic bedding materials *in vitro*, and extend these observations into practical advice for regional dairy farmers.

Researchers at the University of Minnesota (MN) will continue to develop and evaluate different strategies for applying successful and cost-effective selective dry cow therapy programs, as an alternative to blanket dry cow therapy. Applied properly in appropriate herds, selective dry cow therapy offers an opportunity to significantly reduce antibiotic use at dry off while maintaining and promoting udder health. One arm of this research program is to evaluate the accuracy and practical considerations of using different on-farm rapid diagnostic systems (e.g. direct tests: milk culture; indirect tests: milk leukocyte differential counts, somatic cell counts, enzymes, other) for identifying infected cows or quarters that should be treated with an antibiotic in a selective dry cow therapy program.

The University of Missouri (MO) is aiming to explore the effects of intramammary antimicrobial usage on the fecal microbiome and resistome.  This will include exploring if an increase in pathogenic bacteria are found in the feces after the administration of intramammary antibiotics, which could be a concern for dairy food safety.  Future work will also identify if antimicrobial resistance patterns of fecal pathogens are affected by intramammary antibiotic administration.  The University of Missouri is also evaluating antimicrobial peptides as potential therapeutics for diseases of cattle.

Mastitis caused by *S. aureus* requires these bacterium to form complex communities called biofilms. Biofilm formation is necessary for *S. aureus* pathogenesis. Scientists at Rutgers University (NJ) have recently found that the addition of a small molecule that stimulates cellular respiration can disperse *S. aureus* biofilms that had formed in low oxygen environments such as the udder of mastitic cows. They are now trying to determine if such a small molecule can disperse *S. aureus* biofilms in models of infection. Such a finding could lead to new strategies to control or present mastitic infections.

In collaboration with all other scientists at the University of Maryland (UMD), researchers currently investigate alternative therapeutics (n=2) for the treatment of environmental bovine mastitis to reduce usage of currently approved antimicrobials.

At the University of Tennessee (TN), scientists will evaluate the role of internal teat sealants with or without antibiotics in dairy heifers during times of projected wet, muddy conditions in the Southeast to minimize the risk of IMI.

Casein hydrolysate (CH) intramammary infusion for cessation of lactation in one chronically mastitic mammary quarter in dairy cows is being studied in Utah and Idaho. Total milk lost per cow (14%) after the mastitic quarter was involuted, recovery of treated quarters’ milk production after the cows calved again (24% of total-cow milk), and reduction in total cows’ milk SCC after infusion (decreased by approximately 1,000,000/mL) all suggested that this can be an alternative method of drying off one mastitic quarter with a good prospect for return of that quarter’s production following the next calving. Future work includes study of CH as an adjunct, or possible replacement for dry cow antibiotic treatment with or without teat sealant at time of dryoff. Mechanistic studies of mammary involution and immunity following CH will involve collaboration with other laboratories.

Based on the developed disease detection models, researchers at Virginia Tech University (VA) will examine early intervention strategies for clinical mastitis in an effort to reduce antimicrobial usage.

Scientists at the University of Vermont (VT) will quantify the antibacterial activity of potential alternative therapies including plant-derived essential oil products. In addition, scientists will characterize the microbial community structure of bovine teat skin with a focus on the potential antagonistic interactions between *Staphylococcus* species that are either opportunistic mastitis pathogens or normal commensal organisms with the goal of identifying potential beneficial commensal organisms.

Canada: International members will 1) investigate quarter-level selective dry cow therapy to reduce use of antimicrobials on dairies while maintaining udder health (Dufour and Keefe); and 2) describe the current use of antimicrobials on dairies, investigate producers and veterinarians’ motivations for using them, and develop and evaluate a continuous antimicrobial usage surveillance system for Canadian dairies (Dufour).

**WORK PLANNED FOR THE COMING YEAR, LISTED BY OBJECTIVE:**

**OBJECTIVE 1:** Characterize host mechanisms and pathogenic virulence factors associated with mastitis susceptibility and resistance to improve economic outcomes and animal welfare

Hill Farm Research Station, LSU AgCenter: Organisms isolated from cases of mastitis from goats and cows will continue to be identified and tested for antimicrobial susceptibility.

Oregon State University: determination of the effect of SE-enriched milk on immune function and milk macrophages and liver transcriptome.

University of Tennessee: Regions in the bovine genome have been identified that are associated with phenotypes reflecting the strength of immune response to Streptococcus uberis infection and S. uberis concentrations in milk following intramammary challenge. These regions have provided several novel candidate genes that will be examined in the upcoming year for their role in mastitis resistance and their potential as novel targets for mastitis control.

University of Maryland: Examine the effects of Omnigen on the host response to *S.uberis* bovine mastitis.

University of Tennessee: Evaluate the protective effect of conserved immunodominant Staphylococcal surface proteins as vaccine antigens against bovine Staphylococcus aureus mastitis. Analyze data and prepare manuscript for submission.

University of Maine:

Re focus investigations based on current regional mastitis pathogens and antimicrobial susceptibility patterns, informed by our service laboratory work.

**OBJECTIVE 2:** Assess and apply new technologies that advance mastitis control, milk quality and/or dairy food safety.

University of Montreal: 1) to complete a review and meta-analysis on use of non-antimicrobial approaches for dry-off treatment. This meta-analysis will help organic producers tackling udder health issue. 2) To complete the analyses for a study assessing the impact of quarter-based selective dry cow treatment on udder health and use of antimicrobial at drying off. 3) To study the impact or recycled manure bedding on udder health and milk microbiota in the context of Canadian herds. 4) To complete data collection for a project aiming at quantifying antimicrobials use and bacterial antimicrobial resistance on dairies and at developing a surveillance system for these.

Cornell University: Validation of the systems dynamic model which can optimize antimicrobial use in treatment and prevention of mastitis in typical U.S. Dairy Farms. Estimation of economic value of antibiotics used in prevention and treatment of mastitis in U.S. Dairy farms.

Mississippi State University: Three project are in the process of being conducted. The first involves evaluating the adoption of on-farm bacteriological culture, where farmer perception and management decisions will be evaluated. On-farm cultures have been shown to be an effective tool in mastitis management and detection, but the adoption rate has been slow. The purpose of this study is to better understand the human-aspect of this lack of adoption and to see how producers respond to having the supplies and help donated to them for a month instead of having to pursue it on their own. The second study is an intensive two-week study where cows housed on pasture and cows in confinement (on the same farm) will be sampled at each milking (twice daily) to determine differences in milk somatic cell count, components, and bacteriology between milkings, days, and housing groups. The goal of this project is to better understand how much somatic cell count fluctuates between milkings and whether or not pastured animals are more likely to have a higher somatic cell count as is often assumed by industry representatives. The third project is evaluating the effects of heat stress and the use of a center pivot as a cooling source on clinical and subclinical mastitis. Three groups of cows will be evaluated: pasture no shade or cooling, pasture shade, and pasture center pivot. Center pivots are becoming more common as a means of cow cooling on pasture so this study aims at determining what the physiological and behavioral factors are to using that versus other common pasture situations.

University of Minnesota: 1) Godden, S., T. Schoenfus, R. Fink, J. Timmerman, C. Gebhart, E. Royster, S. Wells. Field validation of Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-ToF) for the identification of dairy microorganisms critical for safety and quality. 2) Patel, K., S. Godden, E. Royster, J. Timmerman, B. Crooker, and L. Fox. Investigation of the relationship between bacteria counts, bedding characteristics and bedding management practices with udder health and milk quality on dairy farms (details described above). 3) S. Godden, E. Royster, B. Crooker and S. Rowe. Investigation of the relationship between bedding characteristics and intramammary infection in late lactation dairy cows (details described above). Herd sampling will be repeated during the winter of 2017/2018.

University of Missouri: we will focused on the epidemiology of staphylococcal mastitis in dairy goats and dairy cows. Additionally we will be evaluating milk quality in small holder hand-milked herds in the Amish community in Missouri and cow herds in the Galapagos.

University of Maryland: Continue to follow for dairy producers that have recently transition from conventional to robotic milking systems in the Northeast region.

Hill Farm Research Station, LSU AgCenter: Plant based products will continue to be evaluated for antibacterial activity and tested for their potential activity as mastitis therapeutics.

University of Tennessee: 1) Evaluate antimicrobial resistance patterns of major mastitis pathogens collected from KY, MS, TN, VA as part of the Southeast Quality Milk Initiative (USDA-NIFA 2013-68004-20424). Analyze data and prepare manuscript for submission. 2) Evaluate the occurrence and diversity of antimicrobial resistant bacteria and abundance of resistance genes in dairy farm environments. Analyze data and prepare manuscript for submission.

University of Maine: Investigate a cow-side screening methodology for farms with a history of prototheca mastitis. Continue quality assurance and control efforts to reduce food safety risks posed by mastitis pathogens. Continue investigations of bedding-based environmental control of mastitis pathogens, including a collaboration with UMaine colleague Juan Romero. Continue investigation into pathogenesis and control of Corynebacterium pseudotuberculosis in sheep and goats for dairy and general use.

University of Vermont: 1) Prepare manuscript describing *Staphylococcus* species and strain diversity on farms that make farmstead cheese in Vermont. 2) Prepare manuscript describing antimicrobial susceptibility and biofilm genotypes and phenotypes for staphylococci isolated from farms in Vermont. 3) Prepare manuscript describing *Staphylococcus* species isolates for antimicrobial (bacteriocin) production; 4) Prepare manuscript describing the antimicrobial activities of plant derived essential oil products; 5) Prepare manuscript describing development of MLST scheme for strain typing S. chromogenes isolates. 6) Evaluate the use of core genome multilocus sequence typing to characterize temporal and geographic variation in staphylococcus mastitis associated isolates from Vermont dairy farms

**OBJECTIVE 3:** Identify and apply new strategies associated with the control of mastitis that can reduce the use of antibiotics in dairy herds.

Scientists at the University of Connecticut (CT) will the use of ultrasound-guided intramammary scanning will be investigated as a tool to provide additional information about IMI and tissue damage that can potentially be used to target dry-off treatment as part of a total program to reduce the amount of antibiotic use in dairy cattle.

The NE-1048 research group at the University of Maine (ME) will assess the impact of paper mill lignin byproducts (PMLBs) as conditioners for dairy bedding, in an effort to help develop products to reduce the incidence of mastitis without additional use of antibiotics. Maine will evaluate selected PMLBs against major mastitis pathogens (bacterial and fungal) *in vitro*, assess their efficacy in the context of commonly used organic bedding materials *in vitro*, and extend these observations into practical advice for regional dairy farmers.

Researchers at the University of Minnesota (MN) will continue to develop and evaluate different strategies for applying successful and cost-effective selective dry cow therapy programs, as an alternative to blanket dry cow therapy. Applied properly in appropriate herds, selective dry cow therapy offers an opportunity to significantly reduce antibiotic use at dry off while maintaining and promoting udder health. One arm of this research program is to evaluate the accuracy and practical considerations of using different on-farm rapid diagnostic systems (e.g. direct tests: milk culture; indirect tests: milk leukocyte differential counts, somatic cell counts, enzymes, other) for identifying infected cows or quarters that should be treated with an antibiotic in a selective dry cow therapy program.

The University of Missouri (MO) is aiming to explore the effects of intramammary antimicrobial usage on the fecal microbiome and resistome.  This will include exploring if an increase in pathogenic bacteria are found in the feces after the administration of intramammary antibiotics, which could be a concern for dairy food safety.  Future work will also identify if antimicrobial resistance patterns of fecal pathogens are affected by intramammary antibiotic administration.  The University of Missouri is also evaluating antimicrobial peptides as potential therapeutics for diseases of cattle.

Mastitis caused by *S. aureus* requires these bacterium to form complex communities called biofilms. Biofilm formation is necessary for *S. aureus* pathogenesis. Scientists at Rutgers University (NJ) have recently found that the addition of a small molecule that stimulates cellular respiration can disperse *S. aureus* biofilms that had formed in low oxygen environments such as the udder of mastitic cows. They are now trying to determine if such a small molecule can disperse *S. aureus* biofilms in models of infection. Such a finding could lead to new strategies to control or present mastitic infections.

In collaboration with all other scientists at the University of Maryland (UMD), researchers currently investigate alternative therapeutics (n=2) for the treatment of environmental bovine mastitis to reduce usage of currently approved antimicrobials.

At the University of Tennessee (TN), scientists will evaluate the role of internal teat sealants with or without antibiotics in dairy heifers during times of projected wet, muddy conditions in the Southeast to minimize the risk of IMI.

Casein hydrolysate (CH) intramammary infusion for cessation of lactation in one chronically mastitic mammary quarter in dairy cows is being studied in Utah and Idaho. Total milk lost per cow (14%) after the mastitic quarter was involuted, recovery of treated quarters’ milk production after the cows calved again (24% of total-cow milk), and reduction in total cows’ milk SCC after infusion (decreased by approximately 1,000,000/mL) all suggested that this can be an alternative method of drying off one mastitic quarter with a good prospect for return of that quarter’s production following the next calving. Future work includes study of CH as an adjunct, or possible replacement for dry cow antibiotic treatment with or without teat sealant at time of dryoff. Mechanistic studies of mammary involution and immunity following CH will involve collaboration with other laboratories.

Based on the developed disease detection models, researchers at Virginia Tech University (VA) will examine early intervention strategies for clinical mastitis in an effort to reduce antimicrobial usage.

Scientists at the University of Vermont (VT) will quantify the antibacterial activity of potential alternative therapies including plant-derived essential oil products. In addition, scientists will characterize the microbial community structure of bovine teat skin with a focus on the potential antagonistic interactions between *Staphylococcus* species that are either opportunistic mastitis pathogens or normal commensal organisms with the goal of identifying potential beneficial commensal organisms.

Canada: International members will 1) investigate quarter-level selective dry cow therapy to reduce use of antimicrobials on dairies while maintaining udder health (Dufour and Keefe); and 2) describe the current use of antimicrobials on dairies, investigate producers and veterinarians’ motivations for using them, and develop and evaluate a continuous antimicrobial usage surveillance system for Canadian dairies (Dufour).

**PUBLICATION LIST:** use as much space as necessary to complete the publication list below.

**Peer-Reviewed Literature**

University of Montreal

Skoulikas, S., S. Dufour, D. Haine, J. Y. Perreault, and J. P. Roy. Accepted. Early lactation extended pirlimycin therapy against naturally acquired Staphyloccoccus aureus intramammary infections in heifers: A randomized controlled trial. Journal of Dairy Science.

Gaudreau, A. M., J. Labrie, C. Goetz, S. Dufour, and M. Jacques. Accepted. Evaluation of MALDI-TOF MS for the identification of bacteria growing as biofilms. Journal of Microbiological Methods.

Haine, D., D. T. Scholl, I. R. Dohoo, and S. Dufour. Accepted. Diagnosing Intramammary Infection: Controlling Misclassification Bias in Longitudinal Udder Health Studies. Preventive Veterinary Medicine.

Francoz, D., V. Wellemans, J. P. Dupré, J. P. Roy, F. Labelle, P. Lacasse, and S. Dufour. 2017. Invited review: A systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. J Dairy Sci.

Francoz, D., V. Welllemans, J. P. Roy, P. Lacasse, A. Ordonez-Iturriaga, F. Labelle, and S. Dufour. 2017. Non-antibiotic approaches at drying off for treating and preventing intramammary infections: A protocol for a systematic review and meta-analysis. Animal Health Research Reviews:169-175.

Cornell University

Troendle, J., L. W. Tauer, Y. T. Gröhn. 2017. Optimally achieving milk bulk tank somatic cell count thresholds. J. Dairy Sci. 100:731-738.

Kaniyamattam, K., A. De Vries, L. W. Tauer, and Y. T. Gröhn. 2017. Genetic, technical and financial performance of dairy herds achieving optimal milk bulk tank somatic cell count reductions through various voluntary culling strategies. J. Dairy Sci. (under review).

Mississippi State University:

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University of Minnesota

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Godden, S., E. Royster, J. Timmerman, P. Rapnicki and H. Green. 2017. Evaluation of an automated milk leukocyte differential test and the California mastitis test for detecting intramammary infection in early and late lactation quarters and cows. J Dairy Sci. 100:6527-6544

University of Missouri

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Utah State University

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University of Vermont

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Cornell University

Kaniyamattam, K., A. De Vries, L. W. Tauer, and Y. T. Gröhn. 2017. Impact of culling for SCC, milk revenue and estimated breeding values on herd performance. Proceedings of 2017 American Dairy Science Association annual meeting, Pittsburg, Pennsylvania.

Mississippi State University:

Keefer, R.D., J.G. Maples, A.E Stone. The economic feasibility of implementing center pivots on Mississippi dairy farms. 2017. Mississippi Academy of Sciences Summer Symposium. Stoneville, MS.

A.N. Rulewicz, K.B. Graves, R.D. Keefer, A.E Stone. Evaluating the effects of Vista Pre-T on feed efficiency in heat-stressed dairy. 2017. Mississippi Academy of Sciences Summer Symposium. Stoneville, MS.

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A.E. Stone, B.W. Jones, L.M. Mayo, I.C. Tsai, Y.M. Chang, and J.M. Bewley. 2017. Evaluation of activity, feeding time, lying time, rumination time, reticulorumen temperature, and milk yield, conductivity, lactose, protein, and fat to detect subclinical mastitis. 2017. Abstract 71194. Dairy Science Association Annual Meeting. Pittsburgh, PA.

B.W. Jones, L.M. Mayo, I.C. Tsai, A.E. Stone, Y.M. Chang, and J.M. Bewley. 2017. Detection of lame cattle using behavioral and physiological changes as measured by precision dairy monitoring technologies. Abstract 355. American Dairy Science Association Annual Meeting. Pittsburgh, PA.

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University of Minnesota

Investigation of the relationship between bacteria counts, bedding characteristics and bedding management practices with udder health and milk quality on dairy farms: preliminary results. American Association of Bovine Practitioners Annual conference, Omaha, NE, USA. September 14-16, 2017

Investigation of the relationship between bacteria counts, bedding characteristics and bedding management practices with udder health and milk quality on dairy farms: preliminary results. Minnesota Dairy Health Conference, Saint Paul, MN, USA. April 11-13, 2017

University of Missouri

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Bernier Gosselin V, Middleton JR, Zhang M. 2017. Diagnostic performance of a competitive ELISA using frozen-thawed milk for identification of SRLV-infected goats. Proceedings of the 40th Annual CVM Research Day (Phi Zeta). 5 May 2017. Abstract #9 – 2nd and 3rd Year Resident & Graduate Student Oral Presentations.

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Utah State University

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Cornell University

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University of Minnesota

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University of Missouri

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Michigan State University

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Hovingh, E. P. and R. Moore-Foster. Milking efficiency- As defined by the cow. Workshop presented at the 1st Annual Michigan Dairy Health Symposium, Lansing, MI, February, 2017.

Durst, P., S. Moore, and F. San Emeterio. Engaged employees – What does that mean? Workshop presented at the 1st Annual Michigan Dairy Health Symposium, Lansing, MI, February, 2017.

Thomson, R., and R. J. Erskine. Five minute mastitis hot topics- Teat dips, antibiotics, somatic cell counts, selective dry cow therapy, and others…… Workshop presented at the 1st Annual Michigan Dairy Health Symposium, Lansing, MI, February, 2017.

Erskine, R. J., M. Borek-Stine, and R. Moore-Foster. Engaged employees: The connection between protocols and performance. Short course presented at the 56th Annual Mtng National Mastitis Council, St. Petersburg, FL February, 2017.

Erskine, R.J. Employee management to improve milk quality, seminar presented to the Western Canadian Dairy Seminar, Red Deer, AB, March, 2017.

Erskine, R.J.\* and R. Moore-Foster. Rethinking milking efficiency. Oral presentation at the American Dairy Science Association, Pittsburgh, PA, June, 2017.

Moore-Foster, R. and R.J. Erskine\*. Making the connection between employees and cows for milking protocols. Oral presentation at the American Diary Association, Pittsburgh, PA, June, 2017.

University of Tennessee

Kerro Dego, O., R. D. Abdi, J. Vaughn, C. Merrill, S. M. Cantwell, B. E. Gillespie, R. A. Almeida, S. I. Headrick, G. M. Pighetti, P. Krawczel, J. Keflot, J. M. Bewley and S. P. Oliver. 2017. Antimicrobial Resistance Patterns of Staphylococcus aureus Isolates from Cases of Bovine Mastitis. Proceedings of National Mastitis Council, p 60, 56th meeting January 28 – 31, 2017, Trade winds Island Grand Resort, St. Pete Beach, Fl.

**Poster Presentations**

University of Montreal

Massé, J., S. Dufour, and M. Archambault. 2016. Characterization of Klebsiella spp. isolates from bovine mammary gland infections. in Proc. Congrès de bactériologie intégrative: symbiose & pathogenèse, Québec, QC, Canada.

Kabera, F., S. Dufour, G. P. Keefe, and J. P. Roy. 2017. Quarter-based selective dry-cow therapy using on-farm diagnostics. in Proc. 2017 Canadian Bovine Mastitis and Milk Quality Research Network Annual Scientific Meeting, Montreal, Canada.

Cornell University

Kaniyamattam, K., A. De Vries, L. W. Tauer, and Y. T. Gröhn. 2017. Impact of culling for SCC, milk revenue and estimated breeding values on herd performance. Proceedings of 2017 American Dairy Science Association annual meeting, Pittsburg, Pennslyvania.

University of Minnesota

Investigation of the relationship between bacteria counts, bedding characteristics and bedding management practices with udder health and milk quality on dairy farms: preliminary results. Points of Pride Research Day. College of Veterinary Medicine, University of Minnesota, Saint Paul, MN. October 4, 2017.

Pilot Study: Evaluation of the Effect of Selective Dry Cow Therapy on Udder Health. Points of Pride Research Day. College of Veterinary Medicine, University of Minnesota, Saint Paul, MN. October 5, 2016.

University of Tennessee

Couture VL, PD Krawczel, GM Pighetti, RA Almeida, SP Oliver. 2017. Relationship between the clinical and behavioural response to mastitis challenge with Streptococcus uberis from Holstein dairy cows. American Dairy Science Association Annual Meeting, Pittsburgh, PA, July 2017.

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Merrill C, Reta D. Abdi, Barbara E. Gillespie, Jacqueline Vaughn, and O. Kerro Dego. 2016. Evaluation of the Immune Response and Protection in Dairy Cows Vaccinated with Staphylococcus aureus Surface Proteins. UT Beef and Forage Center Annual Research and Recommendation Meeting, December 13, 2016. Poster presentation.

Vaughn J., R. D. Abdi, Barbara E. Gillespie, C. Merrill and O. Kerro Dego. 2016. Clonal diversity and enterotoxin production patterns of S. aureus isolates from cases of bovine mastitis. UT Beef and Forage Center Annual Research and Recommendation Meeting, December 13, 2016. Poster presentation.

State University of New Jersey

Cerezo, J., Al-Tameemi, H.M., Boyd J.M. Screening the Library of FDA Approved Drugs for Inhibitors of Bacterial Iron-Sulfur Cluster Assembly. Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ, 2017.

Mashruwala A.A., Earle C., van de Guchte A., Boyd J.M. Regulation of Clp proteases by SrrAB in Staphylococcus aureus. Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ, 2017.

Mashruwala A.A., van de Guchte A., Boyd J.M. Cellular respiration as a trigger for multicellular behavior in Staphylococcus aureus. Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ, 2017.

Al-Tameemi H.M., Boyd J.M. Copper Stress in Staphylococcus aureus involves Perturbing Iron Homeostasis. Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ, 2017.

Purdy M., Mohammed N.\*, Crane S., Boyd J.M. Effect of Blue Light on Propionibacterium acnes. Rutgers Microbiology Symposium. Rutgers University, New Brunswick, NJ, 2017.

Cerezo J., Al-Tameemi H.M., Boyd J.M. Screening the Library of FDA Approved Drugs for Inhibitors of Bacterial Iron-Sulfur Cluster Assembly. Aresty Research Symposium. Rutgers University, Piscataway, NJ, 2017.

Al­Tameemi H.M., Roberts C., Mashruwala A.A., Rosario­Cruz Z., Sause W., Torres V.J., Belden W.J., Boyd J.M. Iron Sulfur Protein Assembly: A viable Target for Antimicrobial Therapy in Staphylococcus aureus American Society of Microbiology General Meeting. New Orleans, LA, 2017.

Mashruwala A.A., Gries C.M., Scherr T.D., van de Guchte, A., Kielian T., Boyd J.M. Cellular respiration as a signal for programed cell lysis in Staphylococcus aureus. Gordon Research Conference on Staphylococcal Biology. Waterville Valley, NH, 2017.

Rosario-Cruz Z., Eletsky, Nourhan A., Daigham S., Swapna G.V.T., Szyperski T., Montelione G.T., Boyd J.M. NMR Studies of the CopB Protein of the Arginine Catabolic Mobile Element from Staphylococcus aureus and Bacillus subtilis. Center for Advanced Biology and Medicine Meeting. Rutgers University, Piscataway, NJ, 2017.

**TERMINATION REPORT NE-1048**

**Accomplishments:**

Over the past 5-years of this project, we have studied three areas focused on identifying new means of detecting, preventing, and treating bovine mastitis. Our specific objectives were: (i) characterization of host mechanisms associated with mastitis susceptibility and resistance, (ii) characterization and manipulation of virulence factors of mastitis pathogens for enhancing host defense, and (iii) assessment and application of new technologies that advance mastitis control, milk quality and dairy food safety. Both joint research trials and individual studies have been conducted by NE-1048 and accomplishments (listed by objective) include:

**Objective 1:** Characterization of host mechanisms associated with mastitis susceptibility and

resistance.

Achievements include the dietary supplementation of OmniGen ® (GA), 2,4-thiazolidinedione (OR), retinol-binding protein (RBP; ID) and vitamin E (MD) to improve the host immune response during mastitis. Other major achievements include the negative relationship between severity of negative energy balance and fat mobilization on important inflammatory mediators (MI), the ability of white blood cells to kill invading microorganisms (WA), the negative impact of antimicrobial resistance on the host immune response (NY), the response of peripheral tissues during mastitis (MD, OR), characterizing the nutrient utilization by leukocytes during mastitis (MD), identification of dermal fibroblasts as a model cell to investigate genetic and epigenetic differences between cows in their innate responses to mastitis causing pathogens (VT), and that CXCR1 may be a promising new candidate gene for mastitis susceptibility (TN). Whole genome SNP association studies also have led to a series of new candidate genes that will be evaluated (TN).

**Objective 2:** Characterization and manipulation of virulence factors of mastitis pathogens for

enhancing host defenses.

Major achievements for this objective are 1) the identification of iron-sulfur cluster metabolism as a virulence factor associated with *S. aureus* (NJ); 2) *S. uberis* adhesion molecule (SUAM) is a relevant virulence factor (TN) and 3) certain genes are involved with enhancing antimicrobial resistance of mastitis causing pathogens such as Klebsiella spp. (Quebec), *Escherichia coli* (*E. coli*; NY) and *Staphylococcus aureus* (*S. aureus*; NY).

**Objective 3:** Assessment and application of new technologies that advance mastitis control, milk

quality, and dairy food safety.

Achievements include controlling mastitis via the use of ultrasound scanning to monitor mastitis (CT), the use of an Automated Milk Leukocyte Differential (MLD) Test for detecting IMI (MN), teat dip efficiency trials to reduce the incidence of mastitis (WA), the development of multiple decision support tools aimed at improving milk quality, reducing mastitis and economics (KT), examining alternative therapeutics for the prevention or treatment of mastitis to reduce antibiotic usage (MO, MD, MA, Quebec), continuing outreach efforts to promote better stewardship of antibiotic use on dairy farms (MI, MO, MN, WI) and improving animal welfare via the development of behavioral monitors (KY, VA, MA).

In summary, the current and past five years work conducted within the framework of the NE-1048 (now NE-1748) has resulted in over 150 refereed publications and over 300 presentations at various scientific and stake-holder forums. International visitors and collaborators are often included in these presentations. In addition to the mastitis research workers conference, the NE-1048 members provide new management strategies to reduce antibiotic usage and technology transfer to the scientific community and industry stakeholders. In the last 4 years, members of the project have collectively published multiple book chapters, in excess of 192 peer-reviewed journal articles, over 300 abstracts and proceedings, and presented numerous oral and poster presentations related to mastitis, milk quality, and food safety. Venues for oral and poster presentations have included the National Mastitis Council regional and annual meetings (attendees include researchers, veterinarians, dairy producers, and representatives from industry), Conference for Research Workers in Animal Diseases, American Association of Bovine Practitioners annual meetings, International Dairy Federation meetings, American Dairy Science Association meetings, World Buiatrics Congress meetings, American Society of Microbiology meetings, Conference on Production Diseases in Farm Animals, Plant and Animal Genome Conference, Agriculture and Agri-Food Canada - Food Safety meetings, American College of Veterinary Internal Medicine annual forum meetings, and several regional extension and veterinary continuing education meetings.

We are continuing to build on our past findings to reduce the incidence of mastitis through additional research and extension activities. Mastitis is clearly a multi-faceted disease that will require continued efforts to not only ensure the production of safe, high quality food, but to do so in a sustainable fashion and with continued improvements in dairy animal welfare and reductions the use of antimicrobial drugs.

**Impact Statements**

### Impacts

The following are impacts, listed by objective, of current and previous work conducted during the last 5 years by NE-1048. We focus on the most recent impacts. Multiple stations have contributed to the various objectives and are listed following each sub-objective.

**Objective 1:** Characterization of host mechanisms associated with mastitis susceptibility and

resistance (DE, GA, ID, OH, MD, MI, NJ, NY, OR, TN, UT, VA, VT, WA, WI).

* Improved the host immune response during mastitis via dietary supplements.
* Further characterize the relationship between severity of negative energy balance and fat mobilization on the ability of the host to kill invading microorganisms
* Identified the negative impact of antimicrobial resistance on the host immune response the response of peripheral tissues during mastitis
* Identified genetic and epigenetic differences, including potential candidate genes, between cows and their innate responses to mastitis causing pathogens

**Objective 2:** Characterization and manipulation of virulence factors of mastitis pathogens for

enhancing host defenses (GA, LA, MO, NJ, NY, PA, Quebec, TN, VA,VT, WI).

* Identification of virulence factors associated with mastitis

**Objective 3:** Assessment and application of new technologies that advance mastitis control, milk

quality and dairy food safety (CT, KT, LA, MD, ME, MI, MN, MO, NY, OH, PA, Prince Edward Island, Quebec, Saskatchewan, UT, VA, WA, WI).

* Controlling mastitis via more precise on-farm detection methods
* Identified the use of different teat dips to reduce the incidence of mastitis
* Developed multiple decision support tools aimed at improving milk quality, reducing mastitis and economics
* Examined alternative therapeutics for the prevention or treatment of mastitis to reduce antibiotic usage
* Implemented outreach efforts to promote better stewardship of antibiotic use on dairy farms
* Improved animal welfare via the development of behavioral monitors.