used. To determine the association between teat-end shape (round = semicircular contour, pointed = cone-shaped, flat = square-shaped) and milking characteristics, a separate linear mixed-effects model of a subset of cows was fitted from Farm 2 using 136 cow observations. Model assumptions were assessed by evaluation of homoscedasticity and normality of standardized residuals. To satisfy this assumption, data of the outcome variable LOW was log transformed. Resulting estimates were subsequently back transformed.

Results

Inter-cow differences accounted for 82% of the variance in AFR and 2MIN, while intra-cow variance accounted for 18% of the variance. The variance in LOW was 31% attributed to inter-cow differences, whereas intra-cow variance accounted for 69%. Mean (± SD) AFR (lb/min) was 6.9 ± 1.7 (7.0, 0.2 – 25.7 (median, range)). Mean 2MIN (lb) was 15.5 ± 4.8 (15.5, 0.1 – 38). Mean LOW (sec) was 14.9 ± 16.4 (11.0, 0 – 446).

We observed the highest AFR in cows between 241 to 300 DIM (6.6, 0.1 (LSM, SE)). Milk yield, stage of lactation, parity, milking time, farm, and manual control mode had an effect on AFR ($P < 0.02$). 2MIN was highest in 2nd-lactation cows during the last trimester of lactation (13.5, 0.2 (LSM, SE)). In addition, parity, milking time, milk yield, and DIM had an effect on 2MIN ($P < 0.001$). Primiparous cows spent less time in LOW (20.7, 20.1 to 21.3 (LSM, 95CI)) than cows in lactation 2 (21.6, 21.0 to 22.3 (LSM, 95CI)), 3 and greater (22.8, 22.1-23.5 (LSM, 95CI); $P < 0.001$)). AFR (lb/min ± SE) was increased by 1.9 ± 0.5 ($P < 0.001$), 2MIN (lb ± SE) was increased by 4.5 ± 1.3 ($P = 0.001$), and LOW was decreased by 24% ($P = 0.10$) in cows with flat teat-end shape, compared with cows with pointed teat-end shape.

Significance

While most of the unexplained variability in AFR and 2MIN was associated with differences between cows, the majority of the variability in LOW could be explained by differences within cows. As a consequence, AFR and 2MIN represent valuable metrics for optimizing efficiency of dairy production and understanding the dynamics between cow and milking machine, while LOW can be considered a good tool for evaluating the dynamics between milking routine and cow. Consideration of differences in milking parameters in cows with different teat-end shapes will help to improve parlor efficiency and udder health.

Milk microbiome assessed through 16S rRNA sequencing during antimicrobial treatment of mastitis -- a randomized clinical trial and longitudinal follow up

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Introduction

Clinical mastitis affects 20 to 30% of all dairy cows at least once throughout lactation (Hertl et al, 2014). Mastitis has been reported to be responsible for as much as 80% of all antimicrobials used in dairy farms in Wisconsin (Pol and Ruegg, 2007) and accounts for 16.5% of all diseases identified in dairy cattle the United States (USDA NAHMS Dairy, 2007). Gram-negative bacteria and culture-negative cases represent more than 50% of mastitis diagnosis in New York (Schukken et al, 2009). Contrasting evidence has been presented regarding the beneficial effects of treating cows with clinical mastitis caused by gram-negative pathogens (Schukken et al, 2011; Suojala et al, 2013), whereas treatment of culture-negative cases is not currently recommended. There is increasing concern regarding the use of antimicrobials in food animals and the possible implications in human health, such as antibiotic resistance in pathogens, highlighting the importance of judicious use of antimicrobials in production animals. Specific aims were to: 1) evaluate the effect of antibiotics on cure of clinical mastitis; 2) use high throughput sequencing to assess the microbiome of milk samples from a mastitic quarter and compare it to the microbiome of ipsilateral healthy quarter of cows diagnosed with clinical mastitis caused by either a Gram-negative pathogen or with no bacterial growth on conventional aerobic culture; and 3) evaluate the over-time effect of prolonged antibiotic therapy on the microbiome profile of mastitic milk.

Materials and Methods

Cows diagnosed with clinical mastitis with a gram-negative pathogen or no visible growth on aerobic culture (n=103) were randomly allocated to either treatment group, which received 5 intramammary infusions of ceftiofur hydrochloride at 24-h intervals only on the affected quarter, or an untreated control group. Serial samples were collected from the affected quarter and an ipsilateral quarter for microbiome analysis. The 16S rRNA gene was amplified from genomic DNA and sequenced using the Illumina MiSeq platform. Sequences were processed via the MiSeq Reporter version 2.5 and QIIME version 1.7.0-dev, for classification of reads, quality filtering, and calculation of number of OTUs, Chao1, and Shannon diversity indexes. Microbiome changes occurring over time and in response to intramammary antibiotic therapy were analyzed at the phylum and family levels using JMP Pro 11. The effect of clinical mastitis on the microbiome was assessed through response screening analysis. P-values were adjusted for false discovery rate. The GLIMMIX procedure of SAS was used to assess the effects of clinical mastitis and intramammary treatment in OTU numbers, Shannon, and Chao1 indexes.

Results

In cows diagnosed with *E. coli*-associated mastitis, the relative abundance of *Enterobacteriaceae* was greater in the milk from mastitic quarters when compared to healthy quarters, which presented a more diverse microbiome. No differences in the rate of change of *Enterobacteriaceae* were observed between treatment groups. Milk of mastitic quarters classified as negative culture by standard laboratory methods did not exhibit major shifts in the bacterial population, nor did treatment with ceftiofur hydrochloride result in major shifts in milk microbiome. Intramammary treatment with ceftiofur hydrochloride did not improve clinical and bacteriological cures of mastitis compared with untreated controls in either Gram-negative or culture-negative mastitis cases.

Significance

Next generation sequencing revealed significant differences in the metagenome of healthy and mastitic quarters from cows diagnosed with Gram-negative pathogens. However, the bacterial profile of healthy and mastitic quarters from cows diagnosed with no bacterial growth under aerobic conditions did not differ. There were no differences in clinical cure or relative abundance of the most abundant bacterial family from mastitic samples between treatment and control groups in any of the bacterial groups evaluated.

Longitudinal characterization of mastitis causing pathogens previously identified as "other Streptococcal species", including *Lactococcus*

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Introduction

Identification of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* isolated from milk using standard microbiological methods is very accurate when compared to 16S sequencing (Wyder, 2011). The remaining Gram-positive, catalase-negative cocci (GPCN) found in milk, including species in the genera *Streptococcus, Lactococcus, Enterococcus,* and *Aerococcus*, cannot be easily or economically differentiated using biochemical tests (Fortin et al, 2003). As a result, these pathogens are frequently reported as "*Streptococcus* species," making it difficult to assess the clinical significance of the differing organisms (Fortin et al, 2003). The objective of this study was to compare bacteriological cure, risk of recurrent mastitis, and longevity within the herd among different GPCN organisms on 5 farms in northern New York.

Materials and Methods

Five farms having >20 cows with GPCN intramammary (IMM) pathogens in the summer of 2014, including *Lactococcus lactis*, were enrolled in this study in April of 2015. All milk samples from those 5 farms submitted to Quality Milk Production Services (QMPS) were cultured using standard microbiological methods (NMC Handbook, 1999). All GPCN samples were then speciated using MALDI-TOF (Bruker Daltonics, The Woodlands, TX) technology to confirm the bacteria present. All cows enrolled were resampled 14 to 28 days after the initial sample in order to assess bacteriological cure, if they remained in the herd. Cows identified with GPCN infections were tracked with Dairy Comp 305 records after test day for days-in-milk (DIM) at time of sampling, parity, milk production, and previous and future incidents of mas-