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RELATIONSHIPS BETWEEN SUBCLINICAL MASTITIS TRAITS IN THE PENNSYLVANIA STATE UNIVERSITY DAIRY HERD

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ABSTRACT

Mastitis, or the inflammatory infection of the mammary gland, is a major economic and welfare concern within modern dairy operations. The major causes of economic loss in mastitis-affected cows are decreased milk production, medication costs, loss of dumped milk, labor costs, decreased longevity and associated cull costs.

Scientists have studied the disease in cattle for decades to better understand and reduce incidences of mastitis. Currently, subclinical mastitis is predominantly detected through the use of a linear somatic cell score, which is a function of the number of somatic cells in one mL of milk. However, the total somatic cells that a cow produces in a day also depends on daily milk yield. We derived a new trait of daily somatic cell output, which ranged from 12 million to 440 billion and with a median of 1.99 billion. To determine the effectiveness of the new and traditional somatic cell measures, we utilized proc mixed and proc glimmix analysis to better understand if linear score, total somatic cells, milk yield or a combination of these factors produces the most effective data to predict mastitis within the Penn State dairy herd. During our analysis, we found that the somatic cell score significantly increased one week before the incidence of mastitis and remained there until two weeks after the mastitis event. This trend was followed by a similar increase in total somatic cell count. A small decrease in milk yield was observed one week before and one week after a mastitis event. The best fitting model for mastitis prediction included both somatic cell score and total somatic cell score. Milk yield was not strongly associated with mastitis. Total daily production of somatic cells is a novel trait that should be explored more fully to improve mastitis diagnosis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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Chapter 1

Literature Review

Causes of Mastitis

In 1984, a Canadian review of necropsies (between 1969 and 1983) in mastitis-affected cows revealed the prevalence of different pathogenic organisms responsible for the infection[1]. Out of the 212 cows with clinical mastitis, 79 percent recorded mastitis as the primary cause of death (167). Within the primarily fatal cases, microbial evaluation found that 64 percent were caused by *Escherichia coli* (*E. coli*, 107), seven percent contained *Klebsiella* sp (12) and seven percent contained *Staphylococcus aureus* (*Staph. aureus*, 11)[1]. The remaining 37 cases involved more than one organism. Pathologically, fatal mastitis involving *E. coli* was found to affect an average of 1.8 quarters of the udder with most infections occurring in the first week after calving (50%). The mean age of cows affected by *E. coli* mastitis was 7.1 years[1]. While *E. coli* was the only organism found in 47.3 percent of the reviewed cases, the bacteria was also observed in 22 cases that recovered multiple pathogenic species. *Klebsiella* was only observed alone, affecting 2.1 quarters per cow on average[1]. The mean age of cows infected by *Klebsiella* was 7.3 years. *Staph. aureus* was reported to spread most widely in the mammary gland, affecting an average of 2.4 quarters per cow. *Staph* was also found to affect younger animals with an average age of 4.8 years at the time of infection[1]. Most of these animals were infected within the first week after calving (6 to 7 days). Mixed infections were commonly observed with the majority containing *E. coli*. A 2000 study from Western Michigan University described clinical mastitis as a decreasing

problem with an evolving change in the importance of infecting agents[2]. At the time, *Escherichia coli* and *Streptococcus uberis* were known as the predominant causative agents of mastitis in the United Kingdom. In 2017, *Staphylococcus aureus* was found to be the most frequent causative agent in Norway, infecting 25.4 percent of milk samples[3]. With varying causative agents, treatment that can target each strain of bacteria and personalized programs is desirable.

Economic Impact of Mastitis

Economically, the amount of financial loss to mastitis varies upon the infectious agent. In a 2010 Danish and American Dairy Science Association partnered study, the SimHerd IV program was used to estimate the differences in economic loss between *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Escherichia coli*, coagulase-negative *Staph* (CNS), and *Streptococcus uberis*[4]. Within the simulation, scientists estimated the costs for a 100-head herd with a 35 percent replacement rate. Each type of mastitis was evaluated as individual risk factors. The economic loss of each case of mastitis varied between 149 and 570 European pounds ($192 to $734.51)[4]. The most expensive cases of mastitis were found to be contagious (e.g. acquired via horizontal transfer) rather than environmental pathogens. *Staph. aureus* was found to be the most expensive type of mastitis closely followed by CNS. These were followed by *E. coli*, *Strep dysgalactiae* and *Strep uberis*[4]. Cattle with an unspecified pathogenic case of mastitis were found to cost the farmer less than *Staph. aureus* and CNS but more than *E. coli*, *Strep dysgalactiae* and *Strep uberis*. In an earlier study based upon the Norwegian Cattle Health Recording System, an analogous simulation observed the net returns in farms that experienced multi pathogenic

mastitis[5]. That study found that the net return experienced varied by the ratio and type of organism observed.

Mastitis Detection

Somatic cell count (SCC) and somatic cell scores (SCS) are utilized to determine the presence of subclinical mastitis. In 2007, Østerås defined a high SCC as more than 200,000 cells/mL[2]. In 2011, Schwarz defined a healthy udder to be less than 100,000 cells/mL[6]. Other studies have defined a high SCC as greater than 150,000 cells/mL[7 8]. In 1994, Harmon described the difference between clinical and subclinical mastitis[11]. Clinical mastitis was characterized to have severe udder inflammation and other classic symptoms while subclinical mastitis (SCM) is observed by changes in milk composition for long periods of time. While clinical mastitis is often easily detected within a herd, farms without a mastitis prevention program can easily miss subclinical mastitis signs resulting in an increase of milk penalties and a decrease in overall income.

The detection of subclinical mastitis is difficult in herds that do not test individual cows for SCS. A study attempted to solve this problem using shallow whole genome sequencing of the milk[9]. Shallow genome sequencing is a new technique utilized to observe genetic variation in cattle. The study attempted to utilize simulations and data from three farms to track a high SCC count to a specific cow in dairies containing up to 500 cows from a single milk tank sample. To create the simulation, n (25, 50, 100, 250 and 500) simulated cows were genotyped with m (10K, 50K, or 750K) markers. A simulation with 10,000 single nucleotide polymorphism (SNP) arrays was used to estimate the SCC of cattle from a herd with less than 100 head[9]. Tests for herds with

more than 100 head were also proposed. Within the feasibility test, blood and bulk-tank milk from 133 Holstein cows were genotyped and 17,000 SNPS were fed into the program. Using this approach, correlations between measured and predicted SCC were +0.91[9]. When importing the sample population into a reference population of 750 Holsteins, the correlations increased to +0.97 with all sequence and +0.96 when a 1-fold sequencing depth of the bulk-tank milk was used (1% of the genome was utilized). When preforming the same experiment on a 520 head farm of Holsteins, the correlations dropped to 0.78 with 10,000 SNPS, 0.89 with all sequence and 0.79 when a 1-fold sequencing depth of the bulk-tank milk was used[9]. Somatic cell scores vary between different stages of lactation. To determine the mastitis status of cattle throughout the lactation period, a study defined seven SCC traits to be utilized for Canadian Holsteins[10].

Understanding the mechanisms of decreased milk yield and classifications of mastitis is necessary to decrease the effect of the disease on farm income. These mechanisms aid farmers in determining when and how to treat cows.

Treatment and Prevention of Mastitis

To reduce the prevalence of mastitis, many farmers have resorted to mastitis prevention programs and protocols. Within the modern industry, the primary goal of the protocol is to remove infected animals and prevent the opportunity for infection[12]. In a 1991 survey, 406 Michigan dairy producers were asked about their mastitis protocol[12]. Afterwards, the loss in milk yield due to mastitis was calculated for each herd. The main factors decreasing milk loss were teat dipping, the inclusion of sanitizer in wash water, summer housing for calving and dry cows, clean bedding, milking machine regulator type, the use of alternating pulsators and the average

milk production of the herd[12]. The use of iodine, chlorhexidine and quaternary ammonium-type teat dips had significant marginal annual revenue changes of $13.79, $16.09, and $22.17 per cow per year[13]. Sanitizer in the water decreased the profitability of the farm as coliform bacteria can reach the teat ends after the germicidal activity is gone between milkings. In 1988 and 1989, fifty random Ohio dairy herds were monitored for one year[14]. Milk from clinically mastitic cows was collected before the start of antibiotic treatment. Eighteen different pathogen classifications were identified in culture. Results from this research were used to estimate the cost to avoid a case of clinical mastitis per cow and by organism. Mastitis prevention cost the producer $14.50 per cow annually[14]. The treatment of clinical cases of mastitis on the other hand, cost the producer $37.91 per cow per year. Environmental pathogens were the most common and costly. Cases in which two pathogens were detected accounted for 35.5 percent of mastitis costs. *E. coli* was the second most costly case of mastitis accounting for 23.3 percent of costs[14]. These two were followed by esculin-hydrolizing *Streptococcus* (6.4%), *Klebsiella spp* (5.7%), esculin-negative cyclic adenosine monophosphate (CAMP) negative *Streptococcus spp* (5.1%), *Enterobacter spp* (4.8%), coagulase-negative *Staphylococcus spp* (4.1%), coagulase-positive *Staphylococcus spp* (3.0%), *S agalactiae* (2.5%), and *Bacillus spp* (1.2%). A specific bacterium was unable to be identified in 9.4% of cases. This was hypothesized to represent cases in which multiple pathogens were responsible for the infection[14].

The nutritional status of the dairy herd is vital to preventing mastitis. Poor maternal nutrition reduces influence milk yield and increases mastitis susceptibility[15]. Vitamin E and selenium play crucial roles in the phagocytic activity of immune cells in the udder and can decrease the risk of mastitis[16]. To reduce such risks supplementation by subcutaneous injection at ten- and five-days post calving is recommended[16]. Zinc may also play a role in udder health, although scientific

evidence varies[15]. Oxidative stress and free radical production are commonly associated with mastitis[17]. Clinical and subclinical mastitis are associated with release of free radicals, increased total oxidant capacity and decreased total antioxidant capacity in milk[17]. This increase of oxidative stress can be contributed to the increase of white blood cells such as neutrophils, macrophages, lymphocytes, and eosinophiles in mastitic mammary tissue[17]. Supplementation with antioxidants may help combat oxidative stress and decrease the severity and risk of mastitis[18]. Antioxidants act to protect the body from free radicals by directly scavenging free radicals or through the inhibition of oxidizing enzymes. Beta-carotene may have antioxidant properties and has been shown to maintain mucosal health along with vitamin A[18]. Studies supplementing vitamin A and beta-carotene in hopes of reducing mastitis incidence have had varied results. Within heifers, there are some unique considerations in addition to those made for older animals[18]. Copper can be used to reduce mastitis risk in heifers through its antioxidant properties. Vitamin and mineral requirements of heifers are influenced by growth and body weight relative to mature size[18]. While it appears very fruitful, the specifics of supplements to control mastitis risk in heifers requires more clinical research.

Scientists at the University of Helsinki studied the efficacy of various antibiotic therapies in the treatment of clinical mastitis[19]. Treatments of amoxicillin, prednisone and clavulanic acid cocktails were randomly assigned to a series of 304 udders. The frequency of antibiotic administration was three (3x12) and five (5x12) times every twelve hours. Clinical symptoms such as mammary gland swelling, milk clotting and rectal temperature were observed in each cow in conjunction with a California Mastitis Milk Test to evaluate improvement on days 0, 4, 14 and 21. The study found a higher treatment failure rate in the 3x12 treatment group (28.2 ± 5.0 vs. 13.4 ± 3.6%) and a lack of difference in cure percentage between the two groups (73.3 ±

7.8 vs. 72.0 ± 7.4%)[19]. It concluded that increasing treatment duration significantly decreased the clinical failure rate while causing no change in cure proportion, somatic cell count, or new infection rate.

Antibiotic use is the current predominant treatment for mastitis because they can effectively control bacterial infection of the mammary gland. However, antibiotic resistance has become a topic of concern in human and animal medicine. Microbial pathogens are continuously evolving new ways to resist common treatment methods. A study describes the evolution of *E. coli* as a pathogen in the UK over 40 years[20]. The study found that the ability of *E. coli* to create reoccurring infection could be explained by its adaptation or the existence of new pathogenic patterns.

In 2005, Japan experienced an outbreak of mastitis with methicillin-resistant *Staph. aureus* (MRSA) defined as the causative agent[21]. The genetic backgrounds of the strains in Japan differed from bovine-associated *Staph. aureus Staph aureus* is a dangerous causative agent of mastitis; invading neutrophils and mammary epithelial cells, forming micro abscesses, and promoting biofilm formation. Penicillin resistance levels in *Staph. aureus* differ within countries ranging from 20 to 30 percent to more than 85 percent[21]. A β-hemolysin–converting bacteriophage and immune invasion cluster were observed in the samples, allowing scientists to infer that a human was the initial inducing agent[21]. Most of the observed cases were subclinical. Thirty-one of 78 mastitis-affected cows were found to carry the MRSA bacteria. Upon the implementation of culling procedures, the outbreak was eliminated after five months[21]. As the effects of antibiotic resistance increase, the dairy industry will likely experience similar outbreaks and will have to draft new protocols on how to deal with these infections.

Genetic Selection for Mastitis Resistance

With the increased public concern about the use of antibiotics in animal agriculture, alternative methods to control mastitis are being explored. One option for the dairy industry is to use selective breeding to decrease cattle susceptibility to mastitis. The idea of inherited mastitis resistance has been around since at least 1938[22]. During that time, Ward utilized data from New Zealand dairy cows and found evidence of heritability of mastitis susceptibility. This is supported by wide-spread adoption of genome sequencing that is more economically feasible and faster when compared to proving a bull through daughter records. Currently, thousands of dairy bulls have been sequenced for many thousands of SNPs across the cattle genome; 80K are currently used in US genomic evaluations. As soon as genome sequencing was utilized for the selection of milk traits, scientists began to consider the potential of incorporating disease markers. Lush 1950 observed 27 herds under the definition of mastitis as "abnormal quarters that produce abnormal milk." Forty-two percent of dams and 70 percent of their daughters were found to be susceptible"[23]. The study failed to recognize daughters of susceptible dams under the age of eight that did not develop mastitis. The inclusion of these data would have dramatically decreased the percentages of susceptible daughters to 66 and 44 in one group and 67 and 43 in the second group. The heritability of mastitis was estimated to be 0.4, making it a highly heritable trait. The study concluded that culling should be used to rapidly decrease mastitis susceptibility[23]. Currently, mastitis resistance in cattle is thought of as a polygenic trait with a relatively low heritability. Heritability calculations of mastitis often greatly differ by the definition used to define mastitis as well as the relative sample size utilized. In 1980, the University of Massachusetts calculated the heritability of mastitis to be 0.14 in a herd of 289

Holstein cows[24]. The heritability of mastitis susceptibility also varies by the breed and location of the observed cattle. A recent study estimated the heritability of a Norwegian Red herd to vary from 0.04 to 0.12[25]. A higher heritability for SCC at 150 days in milk (0.14) was observed in Swedish Holsteins and was lower (0.09) in Italian Holsteins[25]. Steine 1998 determined that genetic progress through selective breeding is possible with mastitis susceptibility despite the low heritability of the trait[26].

There are multiple approaches to genetic selection of mastitis susceptibility in dairy cattle that can continue to be explored. In Denmark, the net return of genetic selection was found to be between €0.5 and €42 based on the specific organism selected against[2]. To receive the greatest net return, the Danish study recommended the use of a multi gene selection process to reduce the susceptibility of as many pathogens as possible. Between 1997 and 2007, the genetic trend for udder health in Denmark has increased at an average of 0.3 index units per year (mean = 100, SD = 10) for AI-tested Holstein bulls (Danish Cattle Association, 2009)[2]. Despite this favorable trend, the mastitis incidence of the country has remained unchanged since 2003[2]. The topic was revisited in 2009[27]. This study showed that the selection of pathogen-specific mastitis traits decreased genetic gain when compared to selection of a nonspecific mastitis trait. At the same time, it was shown that when several pathogen-specific mastitis traits are combined within an udder health index, the low heritabilities were counterbalanced by the genetic correlations between the traits (ra = 0.45 to 0.77)[27]. Scientists sought to identify the differences between the expression of specific proteins in mastitic and non mastitic dairy cows[28]. Pooled samples from six udders of experimentally induced mastitic cows were analyzed 0, 81- and 312-hours after the introduction of *S. uberis*. Prepared samples of the milk were labeled with CyDyes® (CyDye 2, 3 and 5) and analyzed using isoelectric focusing and gel electrophoresis[28]. The DiGE gels were

scanned and ImageQuant, ImageJ and DeCyder™ 2D (version 7.0) software were used to carry out 2-D differential analysis and image processing. Biological Variation Analysis (BVA) software (GE Healthcare life sciences, Buckinghamshire, UK) was also used to match spots (qualitatively and quantitatively) between gels[28]. Five hundred and twenty-one protein spots were identified to change significantly throughout intramammary infection in milk[28]. These proteins can be further evaluated as selection criteria and as biomarker candidates to diagnose mastitis earlier.

The relationship between and utilization of SCC in the genetic selection of mastitis resistant cattle was first explored in 1997 by Schutz[22]. At the time, SCS were already collected and evaluated by the USDA to evaluate yield traits (5 million available SCS)[17]. Schutz utilized this data to create linear, 2-trait linear, threshold and Poisson statistical models in order to determine the heritable links between milk yield and SCS. The average lactation length across all observed lactations was 348 days[22]. Within the database, 77% of cows had lactations of at least 270 days before the discontinuation of records. Mastitis incidence was significantly increased during lactations three through five with the lowest incidence occurring during the first lactation (12.2%)[22]. Twenty seven percent of mastitis events occurred between −10 through 10 days in milk (DIM). When evaluating the heritability of mastitis, Schutz's estimates for all data from the linear models ranged from 0.73 to 3.82%[22]. Estimates from the underlying scale were larger ranging from 4.98 to 11.07%. The standard error of the underlying scale was larger than that of the linear models. The largest heritability estimate was the result of defining mastitis as a transposable (TN) trait[22]. The lowest heritability occurred with the utilization of backcrossing (TI). Consequently, the reliability estimates of mastitis heritability were greatest in the TI model (0.56 TI, 0.53 TN, 0.49 WL)[22]. While the heritability estimates were higher in the threshold and

Poisson models, the linear models were found to be more reliable. Despite a strong genetic correlation between mastitis and SCC, relationships between herd-average mastitis and average test-day SCS was found to be minimal. This result can be explained by the difference in the accuracy and completeness of record keeping protocols between farms[22].

Breed Variation in Mastitis Susceptibility

Somatic cell score (SCS) and mastitis resistance varies between the different dairy breeds. In 2017, Holstein cattle were found to have the lowest SCS (SCS: 2.35, BV: -0.23) followed by Brown Swiss (SCS: 2.55, BV: -0.04), Ayshire (SCS: 2.62, BV: -0.03), Jersey (SCS: 2.80, BV: -0.05), Milking Shorthorn (SCS: 2.84 BV: 0.10) and Guernsey (SCS: 3.07, BV: -0.05)[29]. In 1994, Schultz evaluated the predicted transmitting abilities (PTAs) of SCS for bulls with the largest amount of male offspring in each breed. This data found variability between bulls with a large breed impact[22]. The greatest difference between bulls were found in the Holstein breed. The popularity and increased number of bulls contributing to genetic variation was cited as the explanation of this difference[22].

Data from the Council on Dairy Cattle Breeding contains records on the SCS and milk yield for each breed from 1957 to 2017. The Holstein breed exhibits a decreased SCS through the period of 2004 to 2017. Jerseys were found to have a more recent decline in SCS from 2013 to 2017. In recent years (2006 to 2017), the mean SCS for Brown Swiss has been consistently between 2.59 and 2.44. Differences in SCS among breeds could relate to breed mastitis susceptibility, changes in milk yield that dilute SCS, or a combination of both.

Udder Conformation and Mastitis Incidence

Danish scientists estimated the genetic, environmental correlations and heritability of nine confirmation traits and six measures of mastitis incidence in Danish Red (DR), Danish Holstein (DH) and Danish Jersey (DJ) cattle[30]. With the study, scientists utilized the official scores of first lactation cows, for breeding value estimations of their sires to estimate genetic values of fore udder attachment, rear udder width, udder cleft, udder depth, teat length, teat thickness, front teat distance, milking speed and dairy form. Records from 11,306 DR, 60,438 DH and 10,639 DJ cows were utilized in the study[30]. In order to define six mastitis measures, data was obtained from the national Danish cattle database. Records from 84,593 first-lactation DR, 374,191 first-lactation DH and 82,222 first-lactation DJ cows was extracted[30]. Univariate analyses of the data were utilized to estimate heritabilities and bivariate analyses were utilized to produce correlations. Region was incorporated into the model to take environmental factors into account. Between 13.9 and 17.5 % of first lactation cows were found to have been treated for mastitis between days -10 and 50 of lactation[30]. This statistic created an average of 15.5 to 19.5 mastitis treatments per 100 head. The number of treatments increased to 36.1 to 39.6 per 100 head between days -10 and 350[30].

Conformation trait heritabilities coincided with earlier Danish conformation data analysis. Udder depth, front teat distance and teat length had heritabilities of approximately 0.40. Fore udder attachment was slightly lower with a heritability of 0.34. The heritabilities of rear udder width (DR:0.34, DH:0.22, DJ:0.22), udder cleft (DR:0.27, DH: 0.18, DJ: 0.15) milking speed (DR: 0.35, DH: 0.27, DJ: 0.17) and dairy form (DR: 0.38, DH: 0.28, DJ: 0.25) experienced breed variation[30]. The DH heritabilities closely coincided with a 1994 genetic analysis of Dutch

black and white cattle[31]. The heritabilities of udder depth, milking speed, teat distance and udder cleft were found to be lower than that of the Dutch study. The strongest observed genetic correlation was found to be between fore udder attachment and rear udder width and between udder depth and fore udder attachment. Strong correlation between front teat placement and udder attachment were found in DR and DH (DR: 0.56, DH: 0.47, DJ: 0.21)[30]. A significant correlation between udder depth and rear udder width was observed in DJ (0.61). In DR and DJ, longer teats were found to be associated with thicker teats (DR: 0.48, DH: 0.27, DJ: 0.49)[30].

The heritability of mastitis traits was found to be similar across populations. Within breeds, mastitis heritability was between 0.045 and 0.059 for DR, 0.038 to 0.050 in DH and 0.019 to 0.026 in DJ[30]. Overall, mastitis heritabilities were found to be stable regardless of the length of data collection. The six mastitis traits were found to be highly correlated

Genetic correlations between mastitis and conformation traits were variable. All three breed populations displayed a relatively high negative correlation between fore udder attachment and mastitis incidence (DR: -0.4 to -0.47, DH: -0.28 to -0.37, DJ: -0.28 to -0.40), indicating that strong fore udders are associated with less mastitis[30]. The correlation between rear udder width and mastitis were found to be near zero in DR and DH. A negative correlation of – 0.15 to -0.33 was observed in DJ[30]. Correlations between mastitis and udder cleft were found to be moderate in DR (-0.18 to -0.29) and DH (-0.13 to -0.16) with no correlation observed in DJ (-0.08 to 0.05)[30]. Across populations, cows with high udders were observed to require fewer mastitis treatments (DR: -0.50, DH: -0.50, DJ: -0.30 to -0.50). In DR, mastitis and teat length were slightly negatively correlated (-0.08 to -0.17). This correlation was slightly stronger in DH (-0.15 to -0.23) and slightly positive in DJ (-0.18 to 0.02)[30]. Teat thickness was found to be positively associated with mastitis regardless of population (DR: 0.12 to 0.20, DH: 0.25 to 0.35, DJ: 0.04 to

0.27). Correlations between teat distance and mastitis were found to be close to zero in all observed populations. The correlation between increasing milking time and mastitis were observed to increase positively over lactation (DR: -0.08 to 0.07, DH: -0.31 to -0.06, DJ: -0.11 to 0.17)[30]. Dairy form selection was found to be positively correlated to mastitis across breeds suggesting that increased selection for angular and thin cows will result in an increased number of mastitis events (0.29 to 0.54)[30]. All of the traits experienced a low environmental correlation with the largest occurring between udder cleft and depth (DR: 0.10, DH: 0.10, DJ: 0.07)[30].

Application in Other Species

The information gathered from mastitis research in dairy cattle can be utilized to improve mastitis resistance in other production species. Commercial dairy sheep production and use is primarily concentrated in Mediterranean areas. Two thirds of the income of the dairy sheep industry is derived from milk sales[32 33]. Milk yield improvement programs have been in place since 2000 and have been successful due to the moderate to high heritability characteristics of the trait[34]. Scientists evaluated whether a genetic selection program similar to that of dairy cattle could be feasible in sheep[35]. To determine this, milk yield and mastitis resistance records were collected monthly during the lactation cycle of 609 Chios sheep. Each ewe was genotyped for a mastitis specific 960 SNP array. It was determined that there was no relationship between milk yield and mastitis resistance in sheep. Environmental factors favored milk production and the overall health of the udder[35]. Four new Quantitative Trait Loci (QTLs) affecting milk yield were detected on chromosomes 2, 12, 16 and 19 in areas distinct from previously identified mastitis resistance sites. Seven genes (DNAJA1, DNAJC10, FGF10, GHR, HMGCS1, LYPLA1,

OXCT1) located within these regions were highly expressed in the mammary gland and milk transcriptome suggesting that they may play a role in milk production and synthesis[35]. Four additional genes (DNAJC10, FGF10, OXCT1, EMB) were highly expressed in immune tissues. This could suggest that they play a role in the production of milk during a mastitic event. The separation of milk yield and mastitis resistance suggest the ability to select for both without adversely affecting the other[35]. In a second study, the heritabilities for SCC were estimated in sheep[36]. Some previously estimated heritabilities within the study were low, ranging from 0.04 to 0.28 with a weighted average of 0.163[36]. Other studies found that mastitis traits in sheep were moderately to highly heritable[37]. When evaluating breed specific heritabilities for SCC, genetic variation was observed with heritabilities ranging from 0.30 to 0.60[35]. The information gained from these experiments can be utilized to improve milk production and decrease the incidence of mastitis in the meat sheep industry[38]. An increase in yield could theoretically increase gain of the offspring and decrease the days on feed[39]. Reducing mastitis incidence could decrease the number of lambs that require supplementation and decrease treatment costs to the farmer. The exploration of similar studies in dairy goats[40], meat goats[41] and pigs[42] could also increase the amount of available income for the producer.

Summary

Inflammatory infection of the mammary gland, known as mastitis, is the dairy industry's costliest disease. The main causes of economic loss from mastitis are decreased milk production, medication costs, dump loss, labor, decreased longevity and cull costs. The severity of mastitis and impacts on milk composition can vary greatly. When studying both clinical and subclinical

cases of mastitis, changes in somatic cell concentrations provide indicators. Somatic cell scores (SCS) and somatic cell counts (SCC) have become the standard method to diagnose subclinical mastitis in dairy cattle. But before this scale came about, only the clinical, more severe cases of mastitis were detectable.

The current standard for mastitis treatment includes intermammary antibiotic treatment. With an increased focus catering towards the prevention of antibiotic resistance, alternative treatment and prevention measures have been explored. Management strategies focus upon the utilization of sanitation techniques to decrease the number of bacteria that could potentially infect the udder. Another potential strategy is the use of zinc, beta carotene, vitamin E and selenium supplementation to increase the phagocytic activity and decrease oxidative stress within the udder. While these strategies are promising, more research is needed before they can become common practice.

Another promising prevention strategy is the use of genetic selection to select for resistance traits. Thousands of dairy sires have already undergone genomic sequencing for mastitis resistance traits, making the incorporation of mastitis resistance markers feasible. Currently mastitis resistance is considered a polygenic trait with a low rate of heritability, making the selection progress slow. Despite this, strong genetic correlations between mastitis and SCS has been observed over multiple studies. Breed and udder conformation have also been shown to influence SCS and mastitis incidence.

The information gathered from studies on dairy cattle has the potential to be applied towards other production species and vice versa. In a study on dairy sheep, seven genes on chromosomes 2, 12, 16 and 19 were found to influence milk. Yield and four genes were hypothesized to influence milk yield during a mastitic event. The separation of these genes

indicates the potential for resistance selection without compromising milk yield. Small ruminants were observed to have a high heritability for resistance traits when compared to cattle that could suggest a more rapid rate of genetic progress that could improve the yield of lamb crop.

Chapter 2

Introduction

Mastitis or the inflammatory infection of the mammary gland, is the most expensive disease within the dairy industry. The costs of mastitis to the farmer depends on the infectious agent(s) involved and was reported to range from $192 to $734.51 per episode[4]. *Staph. aureus* and other contagious agents were more expensive than environmental causes. In order to properly administer antibiotic treatment, culturing of the causative agent for each cow is recommended[12], which is a strain on labor and management resources. While antibiotics are often effective for the treatment of mastitis, antibiotic resistance has had researchers taking a more sustainable and preventative approach. Although vaccinations are available for some types of mastitis, the majority of mastitis pathogens do not have effective vaccines

Genomic selection is effective at increasing the rate of genetic progress for lower heritability traits such as mastitis resistance[24]. Breeders can select bulls at birth for desirable traits, shortening the generation interval and increasing genetic progress.

Selection for improved udder conformation can help improve mastitis resistance. In a 2000 study, fore udder attachment was associated with a decrease in mastitis in Danish Red (DR), Danish Holstein (DH) and Danish Jersey (DJ) cattle[30]. Cows with high udders were found to have a lower incidence of mastitis, requiring fewer treatments then cows with a deep udder. In contrast, dairy form had a positive correlation with mastitis indicating that mastitis events will increase with an increase in angularity.

In conjunction with genomic selection for mastitis resistance and udder conformation, somatic cell score (SCS) has been used as the standard of subclinical mastitis detection. SCS is a linear measure of somatic cell concentration per ml of milk. Variation in milk yield could alter the relationship between somatic cell score and mastitis status, as there could be a dilatation effect for cows with higher yield. Very few studies have explored the total number of somatic cells cows generate in milk per day. Our study aimed to determine relationships among SCS, milk yield and total somatic cells as a predictor of mastitis. The data collected from this study aims to help producers predict the onset of mastitis in order to preemptively treat the disease prior to the development of clinical symptoms and facilitate the development of additional genetic selection tools to improve resistance to mastitis.

Chapter 3

Materials and Methods

Records from first to seventh lactation Holsteins from The Pennsylvania State University dairy herd from January 2000 through June 2018 were used for this evaluation. Data included 37,035 test day records of 4179 lactations of 1679 cows. Test day records of SCS and milk yield were retrieved from Diary Comp 305 (Valley Ag Software, Tulare, CA) along with mastitis events. Total daily somatic cell (TSC) was derived by multiplying SCC per mL/milk by the daily milk yield in ml (Appendix A). The TSC was not normally distributed, so we used the natural log of TSC (lnTSC) in the analyses.

Test dates were classified according to their proximity to a mastitis event to derive the following mastitis proximity categories: from 8 to 14 days prior to mastitis (-2w; n= 268); 7 to 3 days prior to mastitis (-1w; n= 184); 2 days prior to 2 days post mastitis (0w; n= 238); 3 to 7 days post mastitis (+1w; n= 254); and 8 to 14 days post mastitis (+2w; n= 342). Test days that occurred during a lactation that had mastitis, but not within 14 days (>|2|w; n= 9,123) and that were associated with a mastitis free lactation (none; n= 26,626) were also included.

Two types of analyses were utilized within this study. The first set considered test day milk yield, SCS, and lnTSC as dependent variables with mastitis proximately as an independent effect; the second considered mastitis as the independent variable and the nearest test date records were independent variables. Milk yield, SCS, and lnTSC were evaluated with the MIXED procedure of SAS (v9.4, SAS Institute, Inc, Cary, NC) with mastitis proximity (-2w to +2w, >|2|, none), biweekly classes of days in milk and the lactation number as fixed effects; cow

identification and residual error were fit as random effects. LSMEANS were derived for the effect of mastitis proximity with a tukey adjustment to determine difference among levels.

Mastitis (1=mastitis occurred during a test interval; 0= no mastitis) was evaluated as a binary variable with logistic regression using the GLIMMIX procedure of SAS. Nuisance variables included mastitis proximity, biweekly days in milk class, and lactation (1, 2, ≥3). Regression on milk yield, SCS, and lnTSC were fit individually and jointly to determine associations with the odds of mastitis. Odds of mastitis were then derived for a cow in ≥3rd lactation, the first two weeks of lactation, and milk yield, SCS, and lnTSC at the 10th, 25th, 50th, 75th, and 90th percentile of each variable. An example for lnTSC is demonstrated in Appendix B.

Chapter 4

Results

Descriptive Statistics

Average daily milk yield was 73.95 lbs in first lactation, 86. 24 lbs in second lactation and 92. 36 lbs in ≥third lactation. The average SCC was 132,565 cells in first lactation, 211,663 cells in second lactation and 358,905 in ≥third lactation. The corresponding TSC were 4.1 billion, 7.3 billion and 13.4 billion in lactations 1, 2, and ≥3, respectively. Average SCS was of 2.05, 2.47 and 3.05 in lactations 1, 2, and ≥3, respectively. Most test dates (71.9%) were associated with no mastitis during lactation, 24.6% were associated with a mastitis event outside the range of 2 weeks, and 3.5% were associated with mastitis within two weeks of the test date.

Test Day Analysis

The SCS of mastitic cattle was elevated relative to SCS for cows that did not have mastitis during lactation, or that had mastitis beyond two weeks of the test-date (Figure 1); SCS increased from 3.19 for cows with no mastitis to 5.14 two weeks prior to mastitis detection. There was an additional jump to 7.23 by one week prior to detection and remained higher for weeks 0w, +1w and +2w. Cows over two weeks before or after a mastitis event also displayed an elevated SCS when compared to cows that did not get mastitis during the lactation (4.19). A similar pattern was observed with lnTSC (Figure 2) with lnTSC increasing from 22.1 for lactations with no mastitis to approximately 24.6 from -1w to +2w; this represents a jump in TSC from 4.8 billion cells output in milk per day to 44.0 billion.

Milk yield (Figure 3) was also associated with a mastitis event. Milk yield was 83 pounds for cows not having mastitis, 69.9 pounds for -1w, 80 pounds for 0w, and 72 pounds for +1w;

the increase from -1w to 0w was not expected. Cows with a mastitis event >|2| from a test date had a slightly decreased milk yield when compared to those without a mastitis event (81.3).

Logistic Regression

Calculated odds ratios and 95 percent confidence intervals for a mastitis event at different percentiles relative to SCS in the 50th percentile is described in Table 1. Cows in the 10th percentile experienced odds of 0.38:1.00, whereas those in the 75th (2.25:1) and 90th (5.64:1) percentiles were associated with elevated odds of mastitis. The probability of a mastitis event was observed to be 2.44%, 3.52%, 6.21%, 12.96%, and 27.18% at the 10th, 25th, 50th, 75th, and 90th percentiles of SCC, respectively.

Odds ratios and 95 percent confidence intervals for a mastitis event at different percentiles relative to TSC in the 50th percentile is described in Table 2. The odds for cows in the 10th percentile was 0.40:1.00, whereas those in the 75th (2.12:1) and 90th (5.15:1) percentiles were associated with elevated odds of mastitis. The probability of a mastitis event was 3.40%, 4.74%, 8.03%, 15.60%, and 31.01% at the 10th, 25th, 50th, 75th, and 90th percentiles of TSC, respectively.

The odds ratios and 95 percent confidence intervals for a mastitis event at different percentiles of relative to milk yield is described in Table 3. The odds of mastitis for cows in the 10th percentile was 1.01:1.00, whereas those in the 75th (0.99:1) and 90th (0.97:1) percentiles were associated with decreased odds of mastitis. The probability of a mastitis event was 26.69%, 26.61%, 26.47%, 26.29%, and 26.08% at the 10th, 25th, 50th, 75th, and 90th percentiles of TSC, respectively.

Figure 1. Least-square-means of somatic cell score (SCS) for cows with a mastitis event from two weeks prior through two weeks after a test date, with a test date outside of two weeks from mastitis (>|2|) or that did not have mastitis during lactation (none).

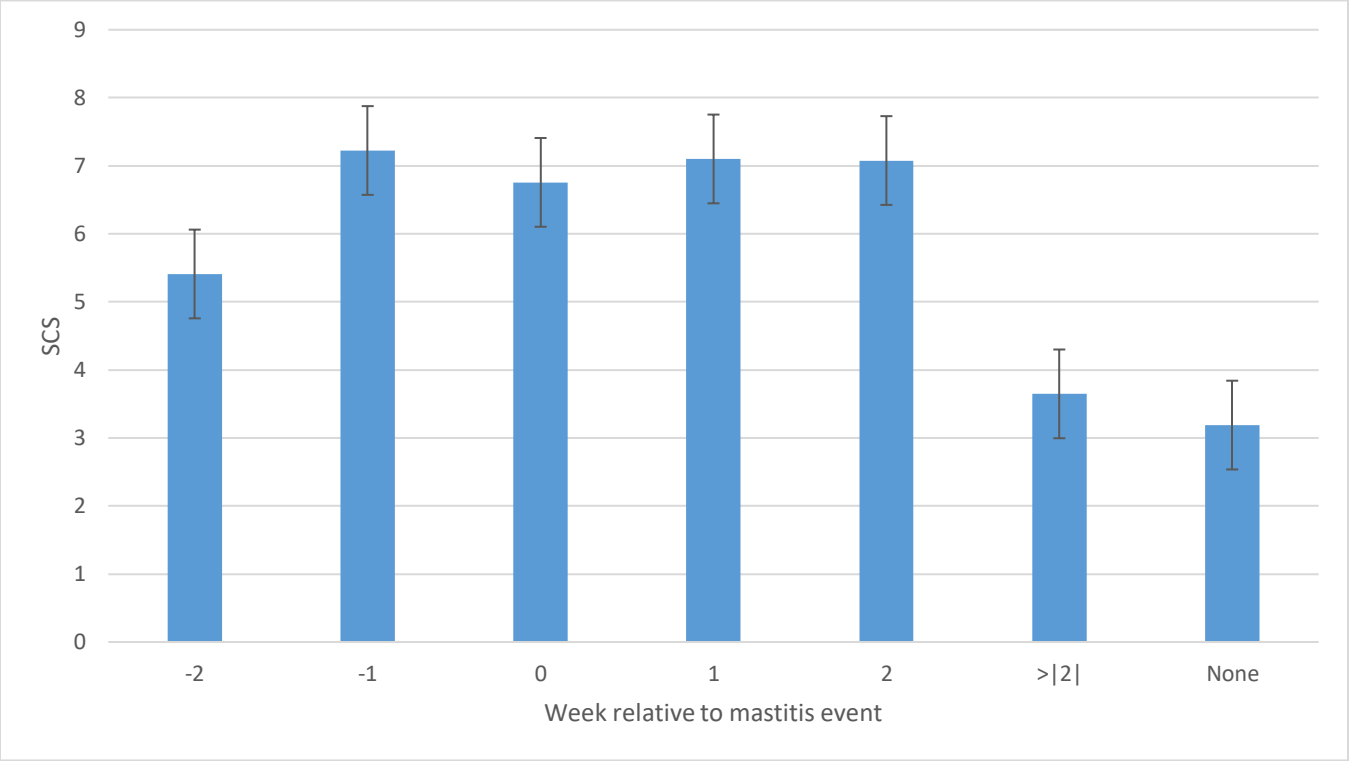

Figure 2. Least-square-means of the log of total somatic cell score (logTSC) for cows with a mastitis event from two weeks prior through two weeks after a test date, with a test date outside of two weeks from mastitis (>|2|) or that did not have mastitis during lactation (none).

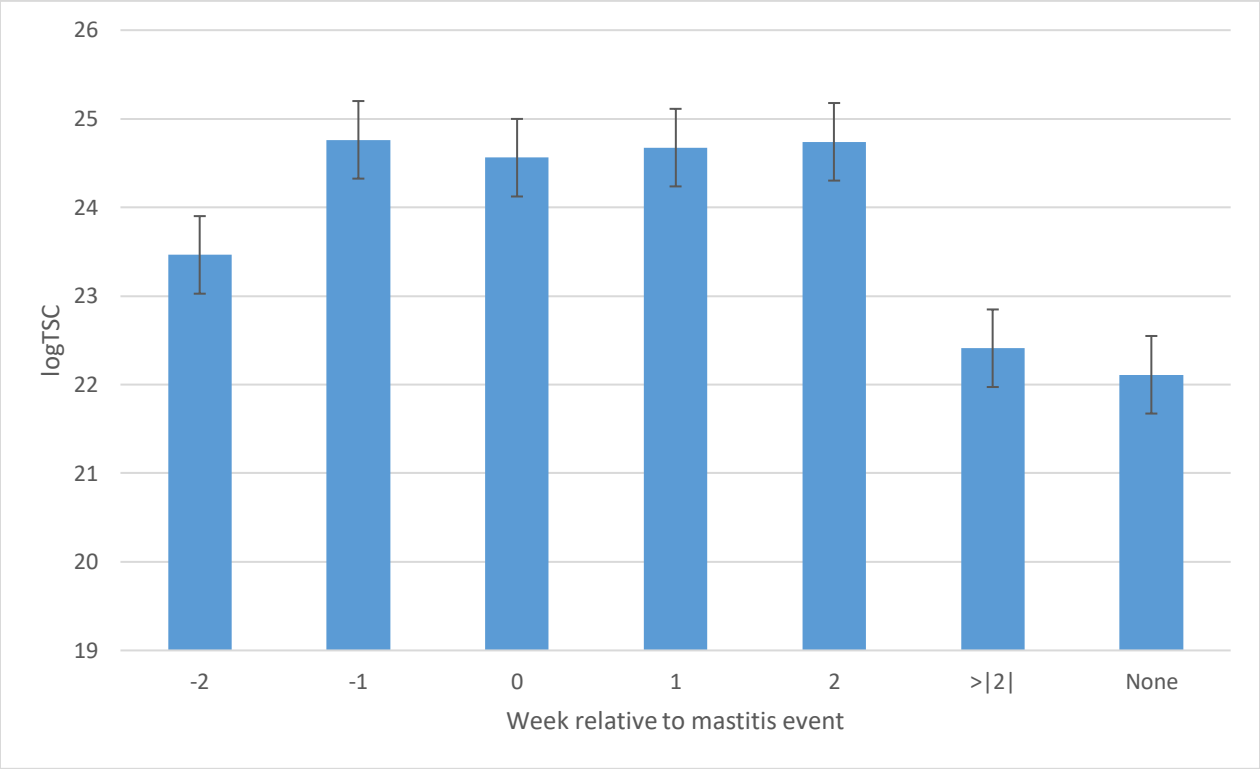

Figure 3. Least-square-means of the milk yield (lbs) for cows with a mastitis event from two weeks prior through two weeks after a test date, with a test date outside of two weeks from mastitis (>|2|) or that did not have mastitis during lactation (none).

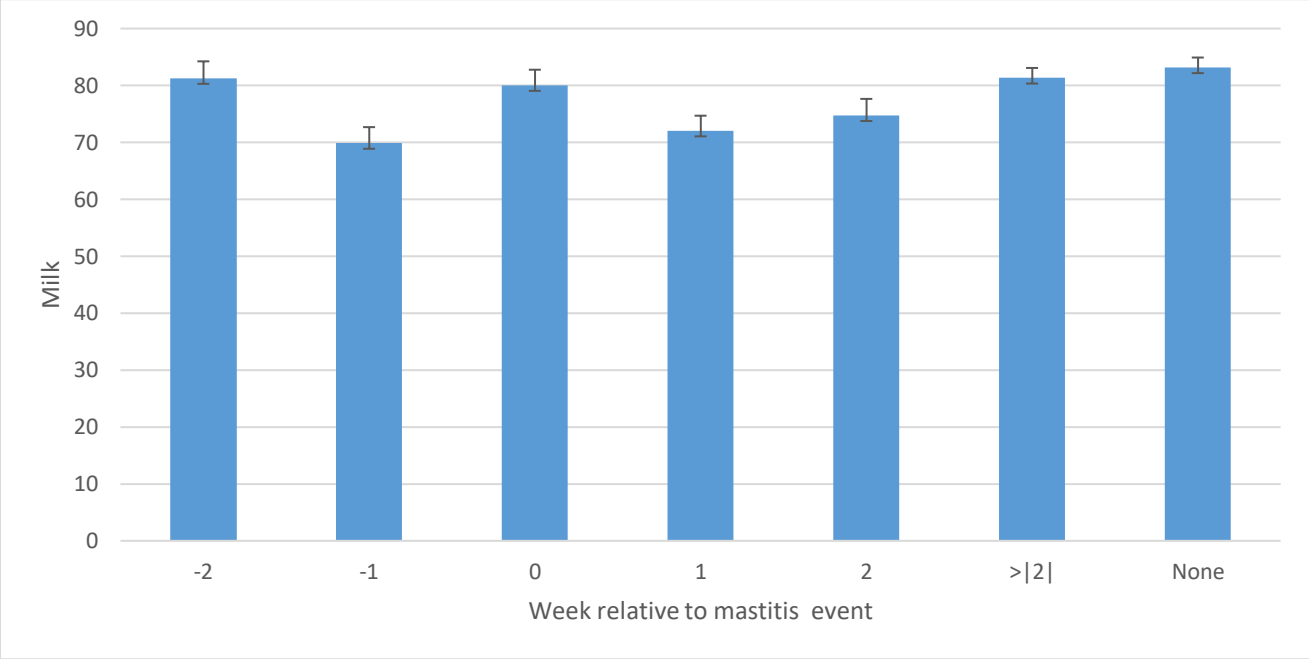

Table 1: Odds ratios of somatic cell score in the 10th, 25th, 50th, 75th and 90th percentiles relative to the 50th percentile with upper- and lower-95 percent confidence limits.

Percentiles	Odds Ratio	Lower Limit	Upper Limit
10	0.38	0.36	0.40
25	0.55	0.54	0.57
50	1.00	1.00	1.00
75	2.25	2.16	2.34
90	5.64	5.16	6.16

Table 2: Odds Ratios of total daily somatic cell score in the 10^{th},25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles relative to the 50th percentile with upper- and lower-95 percent confidence limits.

Percentiles	Odds Ratio	Lower Limit	Upper Limit
10	0.40	0.39	0.42
25	0.57	0.55	0.59
50	1.00	1.00	1.00
75	2.12	2.04	2.20
90	5.15	4.73	5.60

Table 3: Odds ratios of milk yield in the 10th, 25th, 50th, 75th and 90th percentiles relative to the 50th percentile with upper- and lower-95 percent confidence limits.

Percentiles	Odds Ratio	Lower Limit	Upper Limit
10	1.01	1.01	1.01
25	1.01	1.00	1.01
50	1.00	1.00	1.00
75	0.99	0.98	1.00
90	0.97	0.97	0.98

Chapter 5

Discussion

The association of lnTSC with mastitis closely models the relationship between SCS and mastitis within The Pennsylvania State University dairy herd. The relationship between SCS and mastitis has been reported previously and so the relationship between TSC and mastitis was not surprising due to the use of TSC in the SCS calculations. Results concerning relationships between mastitis and milk compositional traits expand upon previous work and practices describing SCS to be indicative of mastitis[2 6 7 8]. The importance of SCS in mastitis detection has been well described within previous literature and our study confirms the importance of SCS in the detection of and selection for mastitis resistance.

One of the questions that arose during this study concerned the influence milk yield had on the SCS of an animal throughout the course of a mastitis event. We hypothesized that a dilution effect may be the cause of the recent genetic decline of SCS in Holstein. Our observations indicate that TSC is more closely related to SCS than milk yield, suggesting that the decline in SCS is mostly not due to dilution of SCS. Nevertheless, the decline in lnTSC (-n%) from -1w to 0w was less than the proportional decline in SCS (-n%) due to increased milk yield. milk yield of the animal in the following days was highly variable. The milk yield of the animal in the following days around mastitis was highly variable.

Milk yield declines may explain a portion of the increase in SCS during a mastitis event. One week before a mastitis event, the SCS was found to be the highest. This correlates with the slight decrease in milk yield observed in Figure 3 one week before a mastitis event. During the week of a mastitis event, milk yield was found to increase to normal levels. While this finding

was unexpected, it correlates with a slight decrease of SCS observed that week. The day after a mastitis event looks like the day before with a decrease in milk yield and increase of SCS. With this trend it can be suggested that milk yield may dilute SCS to some extent and a direct influence on the SCS observed in a milk sample.

Cows with low SCS or lnTSC had much lower odds of mastitis than those in the 75th percentiles and above. This result was expected as animals with a higher SCS and SCC were found to have a higher incidence of disease in previous studies[2 6 7 8]. percentiles and higher. While SCS was found to be the best individual measure of mastitis, the individual measure of TSC was found to follow a similar pattern. In order to account for all variations of mastitis, a combination of SCS and TSC should be utilized as it provided the best model fit.

The odds ratios for milk yield were found to be near 1:1 for all measured percentiles. Due to this, it is safe to assume that a decrease in milk yield alone is not a strong indicator of mastitis.

Chapter 6

Conclusion

Mastitis is an economically devastating infection of the udder observed within animal agriculture. It is especially devastating within the dairy industry due to milk dump loss and a decrease in overall milk yield. To increase the health of the animals and profit margins to the farmers, scientists have been researching ways to prevent and detect mastitis before it shows clinical symptoms.

Currently, SCS is utilized to detect subclinical mastitis. TSC has not been thoroughly evaluated. To determine a relationship between mastitis traits, records of 37,035 test dates from the Penn State Dairy herd were evaluated for milk yield, TSC and SCS. Proc mixed and proc glimmix analyses were utilized to determine correlations between these variables and mastitis incidence. To observe trends, the records were sorted based upon when the cow received a diagnosis of mastitis. The upper and lower odd ratios were then calculated to determine correlations between the variables and mastitis diagnosis.

There are genetic links between TSC and mastitis, suggesting that selection for TSC could be part of genetic selection to increase mastitis resistance. A similar link was observed between SCS and mastitis, confirming the importance of SCS as expected.

Appendix A

Conversions from Somatic Cell Score to Total Somatic Cell Count

The following steps derive TSC and lnTSC for a cow with test-day SCS of 4 and that gives 80 pounds of milk.

1. **Somatic Cell Count (SCC) per mL of milk given Somatic Cell Score**

   $SCC = 2^{(SCS-3)} * 100{,}000$

   Example: SCS = 4

   $SCC = 2^{(4-3)} * 100{,}000 = 200{,}000$ cells / mL

2. **Total Somatic Cell Count (TSC) given Somatic Cell Count (SCC) and pounds of milk yield**

   TSC = SCC*milk produced per day (mL)

   Milk (mL) = 1,000 * kg milk = 1,000 * (lbs milk/2.2)

   lnTSC = ln(TSC)

   Example: SCC = 200000, lbs of milk = 80

   Milk (ml) = 80 lbs/2.2 * 1000 = 36,363mL

   TSC = 200,000*36,363 = 7,272,727,273

   lnTSC=22.71

Appendix B

Odds Ratio Calculations (for lnTSC)

Given the following results from GLIMMIX output:

Intercept = -21.0059, Week 1 solution = 1.7375, lactation ≥3 solution = 0,

lnTSC = 0.7861, standard error of lnTSC = 0.02012, and 5th percentile of lnTSC = 20

LOGIT Calculation

LOGIT = (Intercept + SOLGLIMMIX7 + LGGLIMMIX3 + LOGSC) *5%LOGSC

LOGIT = -21.0059 + 1.7375 + 0 + 0.7861 * 20

LOGIT = -3.5464

Probability of Mastitis

PMAST = EXP (LOGIT)/ (1 + EXP (LOGIT))

PMAST = EXP (-3.5464) / (1 + EXP (-3.5464))

PMAST = 2.80%

Low 95% confidence limit

L95PROB = EXP (-21.0059 + 1.7375 + 0 + (0.7861-2*0.02012) * 20) / (1+ EXP (-21.0059 + 1.7375 + 0 + (0.7861-2*0.02012) * 20))

L95PROB = 1%

Upper 95% confidence limit

U95PROB = EXP (-21.0059 + 1.7375 + 0 + (0.7861+ 2*0.02012) * 20) / (1+ EXP (-21.0059 + 1.7375 + 0 + (0.7861+ 2*0.02012) * 20))

U95PROB = 6.06%

Odds Calculation

ODDS = PMAST / (1-PMAST)

ODDS = 2.80% / (1-2.80%)

ODDS = 0.03

Upper confidence limit Calculation

UODDS = U95PROB / (1-U95PROB)

UODDS = 6.06% / (1- 6.06%)

UODDS = 0.01

Lower confidence limit Calculation

LODDS5% = L95PROB / (1- L95PROB)

LODDS5% = 1% / (1-1%)

LODDS5% = 0.06

Odds Ratio (Median) Calculation

ORM = ODDS 5th percentile / ODDS 50^{th} percentile

ORM = 0.03/0.06

ORM = 0.46

Odds Ratio (Lower 95% confidence limit) Calculation

ORL = LODDS5% / LODDS50%

ORL = 0.06/0.15

ORL = 0.47

Odds Ratio (Upper 95% confidence limit) Calculation

ORU = UODDS5% / UODDS50%

ORU = 0.01/0.03

ORU = 0.44

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ACADEMIC VITA

Hannah Lehew

Skills and Activities

Penn State Student Lion Pride (2019-Present)

- Provides a safe space for the conceptualization, discussion, and execution of matters relevant to LGBTQA student leadership

Undergraduate Honors Research (2018- Present)

- Genetic Correlations and Data Evaluation Using the SAS Program
- Utilized test dates to analyze correlations between mastitis traits

Student's for Cultivating Change (2016- Present)

- Treasurer (2018- Present)
- Collegiate Chapter of the Cultivating Change Foundation. Valuing and elevating LGBT agriculturists through advocacy, education, and community.

Sinai Hospital Surgeons' Teen Liaison (2008- Present)

- Drawing on first-hand patient experiences, act as group and individual mentor/advisor to teens facing orthopedic procedures

Writing Wrongs (2019)

- Published photographs as a part of the book "Outgrowing"

Penn State Collegiate 4-H (2016-2019)

- President (2017- Spring 2019); Secretary (2016-17)
- 2017-18 outreach: PA Farm Show; Operation Gratitude; Puerto Rico Supply Drive; PASLC; Crop Walk; Girls Love Mail

Globe Special Living Option (2016-2019)

- Living environment for students interested in international issues and policy

Work Experience

Fallen Timber Farm (2016- Present)

- Manage a small herd of Registered and Commercial Boer Goats
- Manufacture Goat Milk Products

Traveling Farmhand (2014-Present)

- Work with farmers to perform routine animal care
- Pig, Horse, Poultry, Cattle, Rabbit, Goat and Sheep Experience

Penn State Center for Sexual and Gender Diversity, 101 Boucke Building (Spring 2018)

- Provide resource assistance/referral; conduct resource center staff work
- Implemented a $10,000 grant to create a clothing exchange program for gender-nonconforming students

Honors and Awards

Level four Diamond Clover, Marc David Foundation Award-UP, J. W. Van Dyke Memorial Scholarship, Thevaos Honors Scholarship, 4-H Livestock Skillathon All American