Evaluation of breed, milk production, and udder characteristics on somatic cell count and udder pathogens in lactating Holstein and Jersey cows

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Abstract

Somatic cell count (SCC) is an indicator of the health and cleanliness of dairy cattle and is directly related to incidence of mastitis. The effects of cow breed (n = 10/breed, Holstein & Jersey) on milk yield, milk quality, SCC, bacterial cultures, and hygiene scores were compared over a 6-wk period. Milk Samples with a SCC >350,000 cells/ml were cultured. Holstein cows produced significantly more milk than Jerseys (P = 0.0181) and had a greater conductivity (P = 0.0005). SCC measurements were not significantly different. Jerseys had lower hygiene scores indicating that they were cleaner than Holsteins in their udder and flank area. There were no significant differences in leg scores by breed or bacterial species cultured. The results insinuate that Jerseys are cleaner overall, and that conductivity is related to milk yield.

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Introduction

Mastitis is defined as inflammation of the udder as a result of bacteria entering into the teat of an animal (Taylor & Field, 2016). In dairy cows, it is a common problem. Mastitis is indicated by the presence of somatic, or body, cells that result from the body's attempts to fight infection. Somatic cell counts are a measure that dairy managers use to determine the level of infection either in an individual cow or on a whole-herd basis, as a higher somatic cell count (**SCC**) is indicative of a greater bacterial load for the udder (Sharma et. al., 2011). These bacteria normally enter through the teat sphincter in the housing environment, or when proper precautions are not established in the milking parlor (Barkema et. al., 1999). Mastitis can be divided into two types: subclinical (when the animal only has an elevated SCC and no visual symptoms) and clinical (when the animal exhibits both higher SCCs and symptoms; Harmon, 1994). Symptoms of clinical mastitis include clots in the milk, bloody or pink milk, redness and/or inflamed udders, lowered milk output, lack of appetite, etc. (Eberhart et. al., 1987).

This condition can have many economic ramifications. These include a lowered output of milk, which results in lower profits (Geary et. al., 2013). Chronic cows must be culled from the herd, and the bulk tank SCC becomes elevated, both of which further contribute to economic losses. Bulk tank measurements must remain below 750,000 cells/ml in order to market the milk, but most cooperatives prefer to purchase milk that is below 100,000 cells/ml and producers who consistently sell milk with a lower SCC get better returns for their products than those whose SCC measurements were above 400,000 cells/mu (Geary et. al., 2013). This means that if enough cows have elevated SCCs, then the bulk tank will have to be dumped, wasting milk from healthy cows.

Mastitis can also result in lowered animal welfare due to acute discomfort for the cow. If left untreated, mastitis may lead to certain quarters of the udder becoming unusable, falling off, and even death in extreme cases (Oliveira et. al., 2013). Thus, it is vital that farmers monitor somatic cell counts to ensure that cows are safe and economical.

Many factors can affect SCC and therefore the cow's potential to contract mastitis. By culturing the bacteria in those milk samples with higher somatic cell counts, managers can determine which species of bacteria are infecting their cows. Normally, the cause is either *Streptococcus agalactiae* or *Staphylococcus aureus* (Harmon, 1994). Once the managers determine the specific bacterial culprit, they can administer the proper antibiotics or incorporate more effective management practices to ensure the best health benefits for the cows. They may also use hygiene practices that would discourage the growth of whichever bacterial species is present, as different types of bacteria are caused by different hygienic factors. Furthermore, by examining several physical traits, the dairy managers may discover what traits influence the proliferation of certain strains of mastitis-causing bacteria. Cumulatively looking at the causative relationship data from phenotypic characteristics, SCC, and bacteria species allows dairy farmers to see causative versus correlative study factors, and generates discussion on how to improve both animal health and economic output.

Many relationships exist between SCC and phenotypic traits. Scientists have studied the relationship between udder depth and heightened SCC, or parity and milking speed with SCC, for instance. In a study on the differences between Holstein and Holstein crosses concerning milking speed, milk yield, somatic cell score, and udder measurements, observers found several interesting correlations. They discovered that the faster a cow's milking speed, the less likely she was to contract mastitis and have a heightened SCC (Blottner et. al., 2011). This is because fast-milking cows are not exposed to the milking machines as long as slow-milking cows. Furthermore, deeper udder depths correlated positively with high somatic cell counts (Blottner et. al., 2011). In other words, the closer the animal's udder is to the ground, the closer the proximity of bacteria to the teat sphincter. This results in easier contamination. Other similar studies reveal that breeds which have higher milk yields per cow are more likely to have higher SCC (Sharma et. al., 2011). This is primarily because seedstock producers have been more concerned with breeding for dairy character and high outputs than for disease resistant factors; the correlation is especially prevalent in Brown Swiss and Holstein breeds (Zwald, et. al., 2004).

Despite the importance of breeding for desirable genetic qualities, hygiene is the number one factor that determines the prevalence of SCC for a given dairy operation. Cook and Reinemann (2006) iterate that coliform mastitis could be predicted by the sanitation of the surrounding environment. In four study herds, Ward et. al. (2002) discovered a lower incidence of mastitis and improved hygiene scores in cows kept in a clean environment versus cows in a dirty environment. One such tool dairy operators often utilize to determine the cleanliness of their cows is hygiene scoring. The amount of dirt and manure on the udder is a determinate of this score (set on a point scale of 1 to 4). This scoring applies to the udder, ventral abdomen, lower rear limb, upper rear limb, and tail head (Cook and Reinemann, 2006). It is vitally important that dairy managers remain aware of their cows' hygienic conditions in order to prevent the continuation and spread of mastitis through the herd.

Literature Review

Economic ramifications of elevated SCC

Mastitis and heightened SCC are unprofitable to the dairy industry. Firstly, SCC in excess can result in lower milk quality and possible dumping of milk if greater than 750,000 cells/ml are present. Furthermore, processors can produce fewer milk by-products when the SCC is heightened. Researchers in Ireland examined the impact of mastitis on the industry using causative statistical analysis. Geary et. al. (2013) found that a BMSCC, as it changed from less than 100,000 SCC to over 400,000 SCC, generated approximately 3.2% less revenue per year. Geary et. al. (2013) found that, aside from the obvious reduction in daily raw milk yield that occurred due to mastitis, salvageable milk-solid-based by-products were reduced. They also determined that as bulk milk somatic cell count (BMSCC) increased from <100,000 to >400,000 cells/mL, the quantity of cheese, butter, whole milk powder (WMP), skim milk powder (SMP), and whey powder (WP) that could be produced from the available milk pool decreased (Geary et. al., 2013). The fat and protein recovery of the milk were also diminished significantly (94.12% to 92.70% for fat; 99.91% to 98.76% for protein; Geary et. al., 2013). Thus, by-products of milk created from milk solids decrease significantly when the SCC goes up. Profit is reduced as output is reduced. For instance, in one study, cheese production experienced a minor change with the fluctuation of SCC. Cheese production was found to be lower overall when the cows had high SCC, as opposed to when they had low SCC (Sharma et. al., 2011). A decrease in the cell count from

340,000 to 240,000 resulted in a 1% increase in cheese yield. Additionally, a decrease in SCC from 640,000 to 240,000 resulted in a 3.3% increase.

Yogurt output is also decreased due to the change in protein and fat constituents aforementioned. According to Tamime and Robinson (1999), the lowered recovery of protein and fat has an effect upon yogurt fermentation and can stop this process (Sharma et. al., 2011). A study by Fernandes et. al. (2007) found that higher SCC increases the amount of fatty acids in the yogurt during preservation (Sharma et. al., 2011). Milk with high SCC had reduced heat stability, and thus flocculation can occur during treatments such as evaporation and pasteurization. Therefore, the reduced ability to create yogurt results in reductions in revenue as well. Because processors sacrifice productivity when using high SCC milk for cheese, yogurt, and other milk byproducts, they will not buy from producers who have a record of high SCC in their bulk tank. The cost of antibiotic treatment for cows with high SCC can further add to reduced profit and efficiency. Together, the time and cost factors, coupled with the reluctance of the processors to purchase high SCC milk, can create reductions in economic gain for both processors and producers.

Chemical changes in milk

The milk solids are not only affected by this increase in SCC, but the chemical composition of the milk is also altered, making it unsuitable for human consumption. The alteration of the composition is a direct result of the change in concentration of ions in the raw milk. One study found that electrical conductivity (EC) may be altered due to changes in sodium, potassium, and chloride ion concentrations (Sahu et. al., 2018). Milk from cows infected with mastitis has a significantly higher amount of Na^+ and $Cl^-$with a markedly lower amount of K+. This results in an increase in EC, which is directly proportional to an increase in both udder inflammation and SCC. In other words, milk from cows with mastitis and elevated SCC will have high electrical conductivity. In the study by Sahu et. al. (2018), electrical conductivity was 4.79 at "Normal" levels, 5.47 at "Subclinical," and 7.15 at "Clinical." Subtle changes such as this are at the root of decreased milk quality.

Aside from discovering that increases in SCC result in markedly increased electrical conductivity, Sahu et. al. (2018) found that pH increased with heightened SCC. The milk also became slightly more basic with SCC increases; pH was roughly 6.65 at "Normal" levels, 6.84 at "Subclinical," and 7.11 at "Clinical." The change in pH can result in the milk being inadequate for consumption. Consequently, low milk quality lowers economic gain. However, by using electrical conductivity and pH as tools to measure the SCC in milk, dairy managers can observe the health of their cows and the quality of their milk.

Human Health ramifications

The incidence of mastitis and heightened SCC have more than mere economic ramifications, however. Sharma et. al. (2011) explored the effects of SCC on human health. Somatic cell count affects humans if ingested. First, it most likely contains those pathogenic organisms that caused the somatic cells to be activated and multiply in the first place (Sharma et. al., 2011). One study done by Oliveira et. al. (2013) investigated the types of bacteria that proliferated in certain cases of mastitis across 50 large dairy herds (having >200 head) in Wisconsin. The pathogens commonly recovered in the study were environmental streptococci, *E. coli*, coagulase-negative streptococci (CNS), and

Klebsiella spp. These pathogens can be dangerous especially for those who consume raw milk (Sharma et. al., 2011). However, by pasteurizing the milk, most of the bacteria that can be dangerous for human consumption are destroyed. Nevertheless, though pasteurization reduces the microorganisms considerably, the toxins that the mastitis pathogens produce often cannot be negated by pasteurization. Pasteurization though, is still key as the primary demographic for food-borne illness via milk consumption are those dairy producers who drink unpasteurized milk from their own farms (Sharma et. al., 2011).

Sharma goes on to insinuate that there are zoonotic diseases that can be transferred from dairy cattle to humans via milk consumption and as a result of mastitis in milk. Ingestion of bovine neutrophils is known to cause certain health problems in humans; SCC, of course, heightens the number of neutrophils in the milk (Sharma et. al., 2011). However, a direct connection of SCC to human disease has not yet been found (Sharma et. al., 2011). There is a lack of evidence presented in the literature about exact connections between neutrophils and human health effects. Nevertheless, anyone consuming unpasteurized milk is at risk for food-borne illnesses caused by bacterial pathogens.

Physical traits that affect SCC

Mastitis and SCC are very important to the dairy industry. There are many factors that have been found to contribute to the presence of SCC in dairy herds around the globe. Physiological factors such as udder depth, age, parity, and milking time have all been studied. Furthermore, genotypical factors have been examined, such as the heritability of somatic cell score (SCS). Finally, the greatest predictor of heightened SCC in the dairy herd is the sanitization of the environment. Studies have shown repeatedly that more careful and methodical hygiene practices correlate with lower SCC (Barkema et. al., 1999). In contrast, cattle that were in muddy, humid conditions were far more likely to experience recurring mastitis (Cook, 2002).

The correlation between physiological factors and SCC is interesting. For instance, age is a corresponding factor to heightened SCC, but the direct relationship between the two is not expressly known; age is not thought to be a causative factor in and of itself (Sharma et. al., 2011). The number of quarters affected will also heighten the SCC (i.e. more quarters affected results in higher somatic cell numbers; Sharma et. al., 2011).

Parity is also a predominant physiological factor that corresponds to SCC in a similar manner to age. Cows that are multiparous, having had more than one calf in her lifetime, are at a higher risk of heightened SCC than heifers having their first calves (Sharma et al. 2011). Sharma iterates that the number of lactations has little to do with elevated SCC. However, Sharma et. al. (2011) cite studies by Skrzypek et. al. (2004) wherein producers recorded a marked increase in SCC with "advanced parities."

The stage of lactation also affects the SCC levels in milk. Dohoo and Meek (1982) concluded that SCC will increase later in the lactation curve regardless of the level of infection in the cow. Changes in the ratio of neutrophils to lymphocytes can be observed between the stages of parturition and through the lactation curve (Sharma et. al., 2011). For example, Jensen and Eberhart (1981) concluded that when the cow first gives birth (parturition) SCC could be higher than one million, but in the 7 to 10 days that proceed calving, SCC may drop to 100,000.

It follows, then, that because lactation has such a marked impact on SCC, udder depth and teat conformation are also direct affecters of SCC and mastitis incidence. In examining the association between milk quality and teat and udder traits, Wagay et. al. (2018) performed studies on Tharparker (lower-producing *Bos indicus* breed) cows to evaluate the milk constituents (solids) present. Wagay et. al. (2018) also affirm that, according to the results of a study in Holsteins by Fernandes et. al. (2004), these solid constituents can be linked to SCC since Fernandes's study found lower solids to correspond to higher SCC. The studies done in the Tharparker dairy cows show that longer teats that were closer to the ground contained fewer solids and higher SCC (Wagay et. al., 2018). Teat diameter also had a positive correlation to SCC as larger teat sphincters were subject to greater bacterial invasion (Wagay et. al., 2018). Udder characteristics were also examined in the study; it was found that udder depth becomes a higher positive indicator for SCC as lactation progresses (the results were opposite this finding through the first week after parturition; Wagay et. al., 2018). Deeper udder depths and looser fore-udder attachments, therefore, resulted in higher SCC (Wagay et. al., 2018). This primarily is due to the proximity of the teat sphincter to the ground, making it easier for opportunistic pathogens to enter via mud splashes, walking, bruising, etc. (Cook, 2002; Wagay et. al., 2018).

Physiological factors are important when considering a cow's SCC. Certain characteristics, such as udder depth and teat length, may determine the bacterial exposure of the teat sphincter, to a degree. However, age and parity are also affecters, as older cows with more parities tend to have higher SCC. The physical features of the cow and her conformation, then, have a direct relationship to her health.

Breed and genetic factors that affect SCC

Studies show that higher producing breeds have consistently higher SCC. It follows then, that Holsteins have much higher SCC than lower-producing *Bos indicus* breeds. When compared to Tharparker (1.26 x 10^3 cells/mL), Sahiwal (1.31 x 10^3 cells/mL), Karan Fries (1.61 x 10^3 cells/mL), and Karan Swiss (1.54 x 10^3 cells/mL), Brown Swiss cows (423.31 x 10^3 cells/mL) and Holsteins (310.36 x 10^3 cells/mL) have markedly higher SCC (Singh, 2002; Sharma et. al., 2011). It can be inferred that the higher producing *Bos taurus* breeds have greater tendency than the lower producing *Bos indicus* breeds to have high SCC. However, it is the higher producing varieties of *Bos taurus* (particularly Holstein and Brown Swiss) that are studied in the above comparisons.

Furthermore, because Holsteins have superior milk production to certain other *Bos taurus* breeds, they may be at risk for heightened SCC compared to their lower-producing counterparts. When compared to Friesian cows and Jerseys, Holstein cows yield 12-19% more milk (Coffey et. al., 2016). The yield of Holstein milk solids was also 2% greater than in Jersey cows, and 11% greater than Friesians (Coffey et. al., 2016). However, Coffey et. al. (2016) also found that Jersey cows 15.6% and 17% greater total milk constituents such as percent butterfat and percent protein. Overall, due to their lower producing abilities, Jersey cows may have lower SCC.

The genetics within certain breeds may also have a minor effect upon SCC in adult dairy cows. Although studies have found that predicted transmitting abilities (PTA) are of negligible effect when hygiene is the limiting factor, certain genotypical characteristics make the likelihood of (clinical) mastitis slightly more heritable. It has been shown that somatic cell score (SCS) is somewhat heritable. Somatic cell score is the heritability of SCC (especially as it relates to sires and daughters. An SCC of 100 cells/μl would, for example, convert to an SCS of 3, according to Shook and Schutz (1994). Shook and Schutz (1994) also go on to say that for every 1-unit decrease or increase in SCS, the SCC will be cut in half or doubled, respectively. Somatic cell score is calculated using a base 2 logarithm (Shook and Schutz, 1994). Zwald et. al. (2004) found that mastitis heritability is 0.09 ± 0.01. Somatic cell score heritability and incidence of mastitis also showed a 0.23 correspondence. Albeit, the genetic heritability of mastitis corresponded with (and ultimately resulted from) many other phenotypical PTA characteristics. A study by Rogers et. al. (1991, 1999) concluded that sire PTA has a positive correlation with the PTA for all of the cow's udder traits, especially udder depth (which had a -0.20 correlation). Thus, it can be inferred that selecting for desirable conformation of the udder will reduce incidence of mastitis. Proper udder conformation results in cows that milk out completely, remain clean, and avoid injury (Zwald et. al., 2004).

That being said, the genetic history and PTAs of the cow's sire, therefore, may be noted when selecting for cows that will exhibit lower SCC. Furthermore, when SCC is being measured, researchers need take into account the breed of the animal, as higher producing breeds generally have higher SCC.

Hygienic factors that affect SCC

While phenotypical characteristics are important, their role is far inferior to that of hygiene and good barn management. Hygiene importance cannot be stressed enough. Studies by many researchers over the years have found a positive correlation between good hygienic practices and lowered incidence of mastitis (Neave et. al., 1969; Barkema et. al., 1999; Cook, 2002). Blemishes on the teats result in higher SCC, as does the number of quarters infected (Neave et. al., 1969). In fact, Neave et. al. (1969) go on to say that even simple hygienic routines can reduce infection rates by as much as 45%, and that is regardless of whether teats cups are disinfected between milkings. Farms that used a system of pre-dipping, stripping, and drying before attaching the milking machine could reduce infection by between 45% and 58% (Neave et. al., 1969). By using a pasteurized disinfectant at 85°C, new infections were reduced to 0 in a 1958 trial (Neave et. al., 1969). Good hygiene also involves using iodine tinctures, antibiotic infusion, or dry cow therapy throughout the dry period when the cow is not in the lactating herd; the use of these resulted in lower incidence of infection on the farms studied (Neave et. al., 1969; Barkema et. al., 1999). All of these practices are beneficial to lower incidence of mastitis and subsequently SCC.

Largely, however, it is the cows' environment outside the milking parlor that determines individual SCC. A system of hygiene scoring then becomes necessary to determine the cow's cleanliness upon entering the parlor. The amount of mud, dirt, and debris on the udder and in the near vicinity of the udder is a proven indicator of heightened SCC (Cook and Reinemann, 2006). Cows housed in excessively muddy conditions experienced increased incidence of mastitis than those housed in dry, upkept areas (Cook, 2002). Various beddings and stalling types were examined. The hygiene scores are given to the udder, flank, and leg areas, with 1 indicating the cleanest cows and 2, 3, and 4 indicating increasingly dirty cows (Cook and Reinemann, 2006). The freestall method was more likely to garner high lower leg scores compared to tiestall cows.

However, in the tiestalls, the cows had more debris on their flanks and upper legs, according to Cook (2002). Diagonal lying also results in higher hygiene scores (Cook, 2002). Organic bedding materials, such as shavings, straw, and compost bedded pack barns can all be effective; however, sand is the most comfortable for the animals and results in longer resting times (Cook, 2002). Furthermore, cows on sand bedding have a generally lower SCC because the inorganic nature of the substance discourages bacterial growth (Cook, 2002).

Finally, herd management strategies and the farmers themselves are important to consider when looking at SCC and its correlative factors. In a series of studies and surveys conducted by Barkema et. al. (1999), individual management strategies were observed and matched with BMSCC to observe correlations between hygiene, precision, etc. and BMSCC. Farmers labeled "Clean and accurate" had lower SCC in their cows as opposed to farmers considered "Quick and dirty" in their practices (Barkema et. al., 1999). These "Clean and accurate" farmers also correlated for many hygiene and documentation practices. Hygienic practices included: "Straw removed from calving pen after calving, %," "Manure cleaned from cubicles by hand, frequently >2 times daily," "Years of postmilking teat disinfection," and "Dry cows visually checked for mastitis every day, %" (Barkema et. al., 1999). The wives of the farmers also took the survey; interestingly enough, the farmers whose wives described them as slow and precise in their work (rather than fast) had cows with lower SCC overall (Barkema et. al., 1999). It is wise to note that cows kept in sanitary, "clean and dry," environments will have lower SCC, generally. Also, the management practices utilized, when they are slow and careful, have a direct affect upon udder health and SCC. It is important for the cow and the economic efficiency of the farm that good hygiene is practiced and a methodical cleaning routine followed.

Objective

The objective of this study is to examine the impact of cow breed on milk yield, udder depth, hygiene scores, somatic cell count, and bacterial cultures in the MTSU dairy herd.

Methodology

Dairy cows from the MTSU farm laboratories were utilized for this project (IACUC protocol ID 19-2011). Two groups of cattle (10 Holstein and 10 Jersey; 20 total) were evaluated for a 6-week period to assess the effects of breed, daily milk yield, days in milk (DIM), and udder measurements on SCC and udder pathogens. The cows selected were averaged 102 DIM (days in milk) and 2.5 lactations at the beginning of the study. Somatic cell counts were measured once per week for each of the cows over a 6-week period. SCC were measured by collecting a milk sample while cows were in the parlor for their routine milking schedule (**Figures 12, 13, and 14**). The samples were collected as a composite from all four quarters into a sterile disposable sample bottle labeled with the specific cow's ID (**Figure 15**). Sanitary gloves were worn by the collector, and samples were collected once the teats had gone through the pre-dip, strip, and drying process to ensure the sanitation of the teat end. Rubbing alcohol was applied to the teat end with gauze before collecting the milk sample.

SCC Measurement and Culturing

Samples were measured within 1-2 hours after collection. A few drops of the sample were inserted into a cassette (**Figure 19**) and placed into the DeLaval Cell

Counter (DCC) for measurement of SCC (**Figure 18**). The DCC recorded a SCC measurement within 1 minute. If SCC of the milk sample was greater than 350,000 cells/ml as measured with DCC, the sample was cultured to determine which bacteria were present. Milk samples were placed on a Tri-plate agar media that helps determine which bacterial species were present (**Figure 16**). Sample preparation and culturing techniques were followed according to the Minnesota Easy Culture System User's Guide developed by the University of Minnesota (2013). The three agar mediums on the Tri-plate were as follows: Factor TM media, MacConkey media, and MTKT TM media. These varied mediums helped determine which bacterial species, specifically, were present in a given sample. Samples were stored in an incubator that was maintained at 98.6°F (**Figure 17**). The plate was evaluated at 24 and 48 hours after sampling to record any bacterial growth. The species of bacteria that multiply was then identified as gram positive or gram negative based on the area of growth on the Tri-plate, and potentially ascertained to be *Streptococcus agalactiae* or *Staphylococcus aureus*.

Hygiene Scoring

The cows also were evaluated using a multi-zone hygiene scoring system for udder cleanliness, as shown in **Figure 7** (Cook, 2002; 1= very clean and 4= very dirty). Milking speed, conductivity, and udder measurements were evaluated. Udder measurements taken included udder depth, udder circumference, and teat length. Correlations between milk measurements, udder measurement, hygiene scores, and cow breed were examined. Potentially causative relationships between physical factors and the species of mastitis-causing bacteria were studied. Furthermore, taking breed into account, relationships between breed and SCC were evaluated, as well as phenotypical factors that may heighten SCC. All of these were potential factors determining the likelihood of each of the cows to develop mastitis.

Data Analysis

Production data were downloaded from Afimilk software to record daily milk yield, conductivity, and milking speed. Data were compiled over this 6-week period into a mixed model with repeated measures using breed as the main effect, and analyzed using SAS software (SAS Institute Inc, Cary, NC). Production measures were reported as least squares means including standard deviation with significant differences noted at $P < 0.05$. The Chi-Square test and Proc Freq procedures in SAS were used to evaluate hygiene scores and culture results.

Results

Milk Yield Results

There was a significant correlation between breed and kilograms (kg) of milk given per day (d). Holsteins produced significantly more milk than Jerseys (**Figure 1**), with an average of 37.6 and 26.5 kg/d, respectively (P=0.0004). As was aforementioned, this trend was consistent throughout the study period, even when taking the week-to-week fluctuations of the average into account (**Figure 2**).

Conductivity Results

Differences in conductivity (mS/cm) according to breed were significant (P = 0.0005) (**Table 1**). Likewise, the P-Value according to breed by week remained significant at P = 0.0457. Holstein milk averaged a higher conductivity, at 9.70 mS/cm. Interestingly, this value began at 9.52 and increased with each sequential week; at the end of the five-week study period, the average conductivity for the ten Holstein cows in the study was 9.87 mS/cm (see **Figure 3** and **Figure 4**). Jersey milk had an average conductivity of 8.81 mS/cm, which was significantly lower than that for Holsteins. Unlike the Holsteins, however, this value fluctuated from week to week, beginning at 8.75. The subsequent weeks' average data was as follows: 8.84, 8.78, 8.81, and 8.85 mS/cm.

Hygiene Score Results

In considering the overall hygiene scores according to breed (**Table 2**), more Jersey cows tended to have scores of 1 in the flank and udder areas, with little or no dirt, while more Holsteins had scores of 2 and higher in those areas. The udder and flank areas are the only two areas where the difference between the two breeds was found to be significant (flank: $P = 0.0001$; udder: $P = 0.0003$). It was found that the leg scores by breed had a *P*-Value of 0.1625, which is not significantly different (Table 2). However, the udder scores reported by breed were significantly different ($P = 0.0003$). As can be seen in **Figure 3**, roughly 72% of the cows scoring a 1 were Jerseys, whereas 67% of cows that scored a 2 or higher were Holsteins. However, 69% of the cows with an udder hygiene score of 2 were Holsteins. Scores of 3 and 4 versus all others did not have a significant correlation when considering the udder hygiene scores. Similar to the udder area, the flank area showed a significant correlation, with a *P*-Value of 0.0001. In fact, approximately 61% of Jerseys had a flank score of 1 versus 22% of Holsteins studied (**Figure 4**). 73% of the cows scoring a 1 in the flank area overall were Jerseys, while 62% of the cows scoring a 2 were Holsteins. Also, roughly 85% of the cows scoring a 3 were Holsteins, with the *P*-Value being significant (0.0082). Only one cow scored a 4 in the flank area over the six-week time frame, and she was a Holstein.

Udder Size Results

The udder measurement results are reported in **Table 3**. The *P*-Values indicated in Table 3 for each respective parameter measured did not indicate a correlation between breed and udder measurements. The distance from front to back teats (left) in inches (in), averaged 7.0 ± 0.34 for Holsteins; the same measurements taken in Jerseys averaged 6.3 ± 0.34 in (*P*=0.1489) which indicated no significant effect of breed on left teat distance. Similarly, the distance from front to back teats (right) averaged 6.9 ± 0.28 in for Holsteins and 6.5 ± 0.28 in for Jerseys (*P* = 0.3527). The distance between the front teats (across) averaged 6.6 ± 0.40 in for Holsteins and 5.6 ± 0.40 in for Jerseys (*P* =0.1088). The distance between the back teats (across) averaged 3.1 ± 0.40 in for Holsteins and 3.8 ± 0.40 in for Jerseys (*P* = 0.2358). The total distance along the intramammary groove between the teats, averaged 27.3 ± 0.96 in for Holsteins and 26.3 ± 0.96 in for Jerseys (*P* = 0.4717).

Culture Results

Over the six-week period, 35% of Holsteins were cultured as compared to 25% of Jerseys (0.2320) using the Triplate agar, the results consisted of no bacterial growth (**Figure 8**), *Staphylococcus aureus* (**Figure 9**), other *Staphylococcus* species (not *aureus*) (**Figure 10**), and *Streptococcus* species of bacteria (**Figure 11**). The *P*-Value (0.2320) did not indicate that the difference according to breed was significant when considering whether or not to culture. Similarly, the species of bacteria that grew did not have a significant correlation to breed, both after the 24-hour-observations and 48-hour-observations. The *P*-Values were 0.1568 and 0.2911 after 24 and 48 hours, respectively. **Table 4** indicates the species that grew after the cultures taken from cows with SCC > 350,000 cells/ml were allowed to proliferate (48 hours). 1.7% of both Holsteins and Jerseys showed "no growth"; 5.0% of Holsteins cultured *Staphylococcus aureus*, in comparison to 6.7% of the Jerseys in the study; 21.7% of Holsteins in the study cultured other *Staphylococcus* species, as did 16.7% of the Jerseys; 6.7% of the Holsteins studied cultured *Streptococcus* species, whereas no Jerseys cultured that bacterial genus over the course of the study. In summation, there was little difference by breed in what was cultured and whether or not the animals were cultured in this study.

Discussion

The above results corresponded to several previous studies. The correlation between breed and SCC is seen quite starkly when comparing *Bos taurus* breeds (Holstein, Brown Swiss, Jersey, Guernsey) to the more tropical *Bos indicus* breeds, such as the Tharparker, Karan Fries, and Sahiwal, as in Singh's 2002 study (Sharma et. al., 2011). It was assumed that this was due to higher milk yields, which have been proven to heighten SCS for higher-producing breeds (Sharma et. al., 2011). However, over the five-week period, there was little evidence to correlate average SCC to breed. This could have been because both breeds were of the *Bos taurus* species or possibly to the small sample size. Milk yield, nevertheless, exhibited a marked significance according to breed, as Jerseys are smaller cows with a greater percentage of butterfat in their milk, thus making them lower producers than the Holstein (Coffey, et. al., 2016).

The study on conductivity follows expectations, as milk conductivity bears a direct relationship to SCC, according to the research conducted by Sahu et. al. (2018). They explain that the conductivity of the milk sample will increase as the SCC increases. As such a correlation should have been seen between higher SCC and the breed of the animal, showing that higher-producing breeds have higher conductivity and thus also higher average SCC or SCS. However, the results found no significant correlation between the average SCC and the breed of the animals studied. This misaligns with the extensive research done by Sahu et. al. (2018) on the pH and conductivity of cows with low SCC versus those with high SCC. The differences are explained rather easily, however, because a relatively small sample size was examined in the present study. It follows that, had the study focused on a much larger group, the results would have been more congruent with Sahu's study on pH and conductivity.

The hygiene scores of the cows did not show a significant correlation to SCC. However, this misaligned with the studies performed by Neave, et. al. (1969), which clearly indicate a positive relationship between cleanliness and SCC. The hygiene scores according to breed did vary, however, over the five-week study. The Jersey cows overall were more likely to have a 1 (very clean), while the Holsteins were more likely to have a score of 2 or higher, based on percentages (see **Figure 3** and **Figure 4**). This prompts a study of Holstein activity level, as well as the housing conditions. For instance, the average udder and flank scores were far lower than the leg scores, which is typical and expected in a compost bedded pack barn (Cook, 2002).

In examining the culture results, 35% of Holsteins were cultured and 25% of Jerseys 25%, the majority of *Staphylococcus aureus* cultures were from Jersey cows, while more Holsteins cultured other *Staphylococcus* species or *Streptococcus* species. However, there was no significant difference according to breed in the bacterial species cultured. This fact implies that, while breeds with higher milk yield may have higher SCC, the species of bacteria that grows is not based on such factors. It is, perhaps, more dependent on environmental factors.

Conclusion

Bacterial invasion of the udder is extremely detrimental, not only to the health of the herd, but also to the health of the farm economy and reputation. It is imperative that dairy managers look at the impact on cleanliness in relation to species such as *Staphylococcus aureus*. What is more, the study found that the impact of breed on hygiene score in the udder and flank areas is significant when the animals are housed in a compost bedded pack barn. Holsteins have a significantly greater milk yield than Jerseys, when studied and averaged over a period of weeks. Congruently, the average conductivity of the Holstein milk was higher, indicating a relationship between breed and SCC that the study did not find due to the small sample size (20 cows, 10 Holsteins, 10 Jerseys). Finally, the milk samples taken from these animals, when cultured, indicated no correlation to the breed of cow and the species grown. This implies that an environmental component may have caused the ascertained bacterial species.

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APPENDIX

Table 1. Least squares means and associated standard errors for number of cows, milk yield, somatic cell score, and conductivity of Holstein and Jersey cows.

Measure	**Jersey**	**Holstein**	***SEM***	***P*-value**
No. of cows	10	10		
Milk Yield, kg/d	26.5	37.6	3.01	0.0181
Somatic cell score [a]	14.1	13.4	0.556	0.4417
Conductivity, mS/cm	8.81	9.70	0.15	0.0005*

[a] Somatic cell count data were transformed using the following formula: SCS = log2(SCC/100) + 3.

* Significant difference at $p < 0.05$.

Table 2. Frequency (%) of hygiene score[a] in the leg, udder and flank areas according to breed of Holstein and Jersey cows.

Hygiene Score	**N**	**Holstein (%)**	**Jersey (%)**	***P*-Value**
Leg	118			0.1625
1		3.4	10.2	
2		27.1	35.6	
3		33.9	33.9	
4		35.6	20.3	
Udder	118			0.0003*
1		25.4	64.4	
2		64.4	28.8	
3		8.5	6.8	
4		1.7	0	
Flank	118			0.0001*
1		22.0	61.0	
2		57.6	35.6	
3		18.6	3.4	
4		1.7	0	

[a] According to the method devised by N.B. Cook (2002), hygiene scores are as follows: 1 = very clean; 2 = slightly dirty; 3 = moderately dirty; and 4 = very dirty.

* Significant difference at $p < 0.05$.

Table 3. Least squares means and associated standard errors for udder measurements according to breed for Holstein and Jersey cows.

Measures	**N**	**Holstein**	**Jersey**	**SEM**	***P*-Value**
Left Teat Length, in	20	7.0	6.3	0.34	0.1489
Front Width, in	20	6.6	5.6	0.40	0.1088
Right Teat Length, in	20	6.9	6.5	0.28	0.3527
Back Width, in	20	3.1	3.8	0.40	0.2358
Udder Length Overall, in	20	27.3	26.3	0.96	0.4717

Table 4. Frequency (%) of cows cultured and frequency (%) of bacterial species grown in infected cows compared by breed for Holstein and Jersey cows.

SCC Readings	**Holstein, %**	**Jersey, %**	***P*-Value**
Cows not Cultured [a]	65.0	75.0	
Cows Cultured	35.0	25.0	0.2320
Type of Bacteria			0.2911
No bacterial growth	1.7	1.7	
Staph. aureus identified	5.0	6.7	
Other *Staph*. species	21.7	16.7	
Strep. species	6.7	0	

[a] Milk samples were collected and cultured when SCC reading was ≥ 350,000 cells/ml.

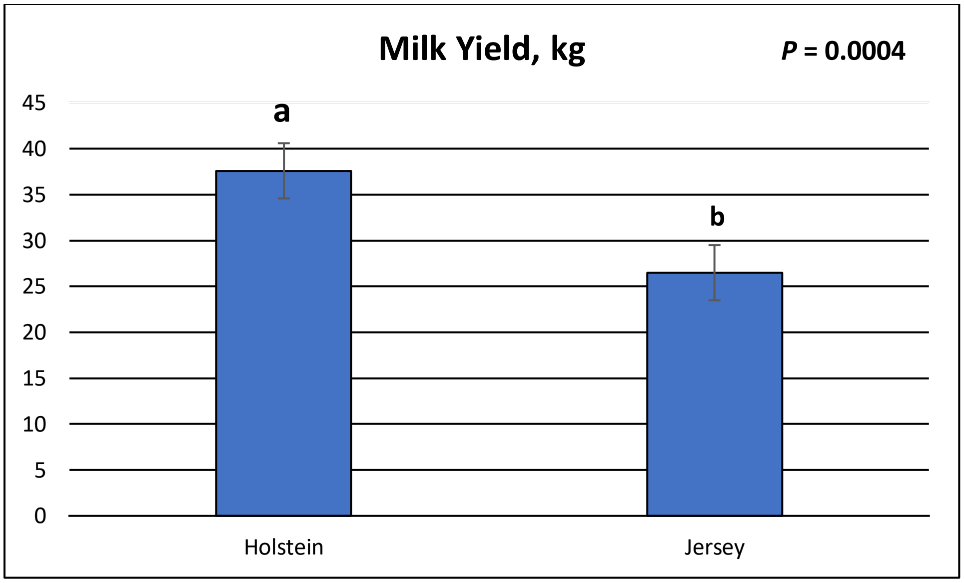


Figure 1. Respective milk yield averages for Holstein and Jersey cows over the 5-wk period. Holsteins averaged 37.6 kg/d, whereas Jerseys averaged 26.5 kg/d.

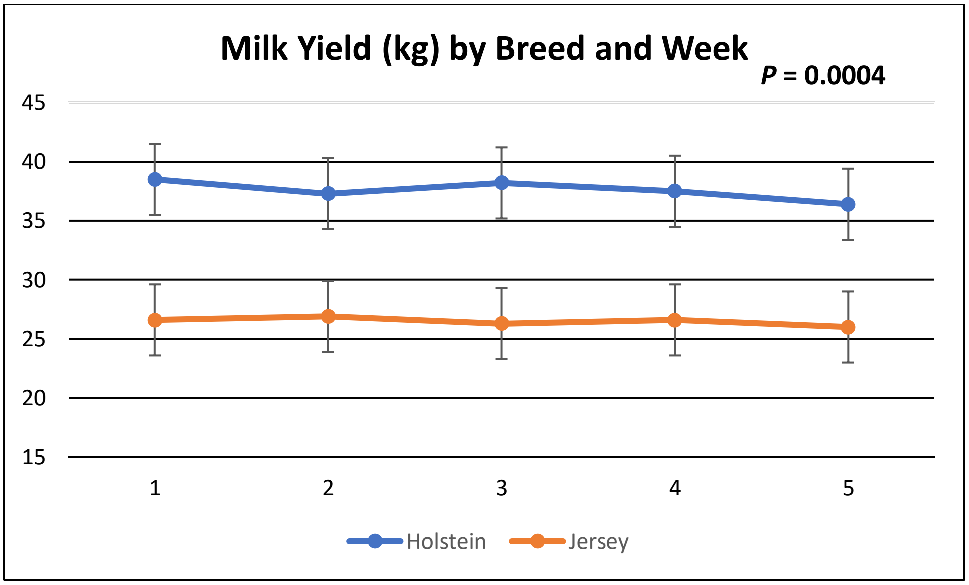


Figure 2. Significant differences according to breed are shown by week. Holsteins averaged ten to twelve kilograms more yield over Jerseys for each measurement taken over the 5-wk period.

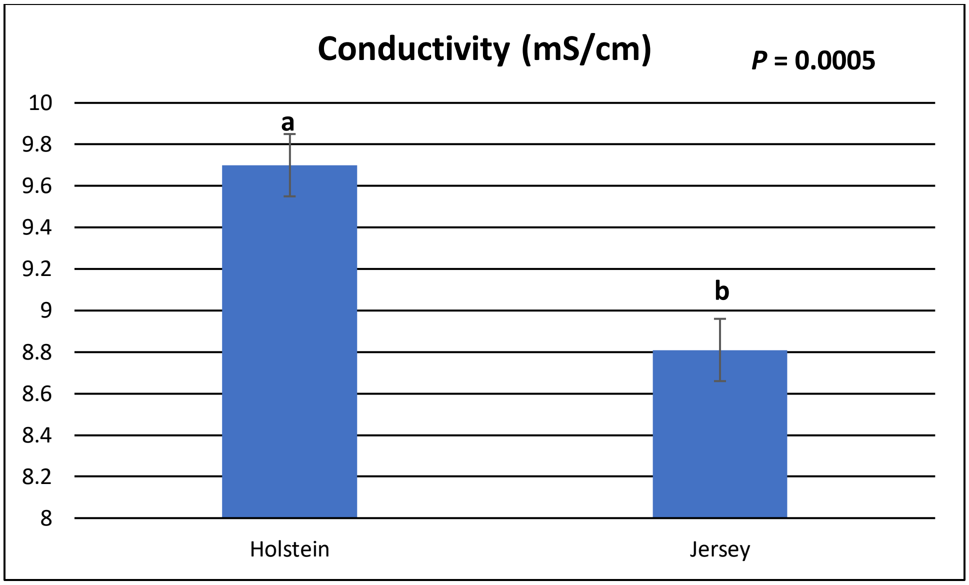


Figure 3. Average conductivity for Holsteins and Jerseys over the 5-wk period. Holsteins averaged 9.70 mS/cm whereas Jerseys averaged 8.81 mS/cm conductivity.

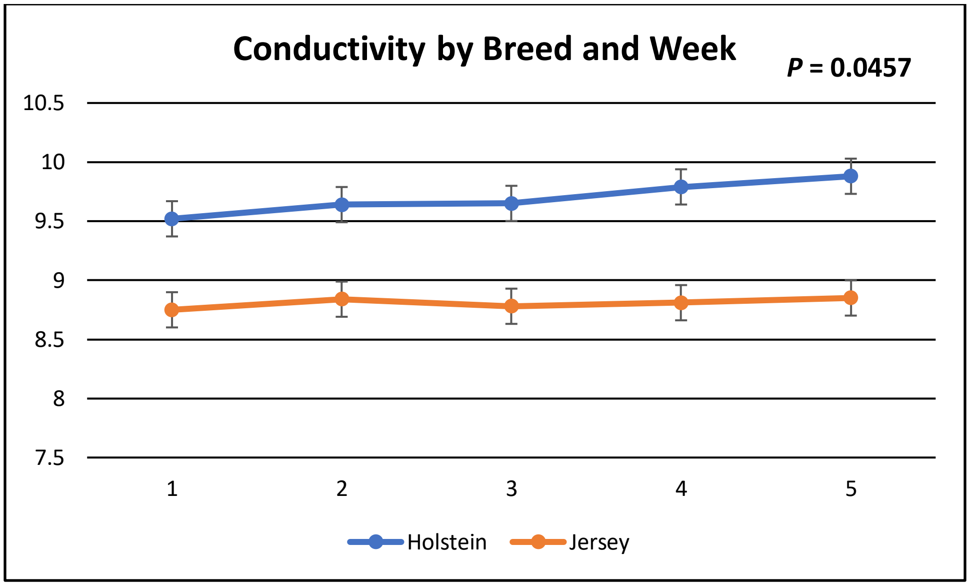


Figure 4. Average conductivity (mS/cm) according to breed are shown by week. Holsteins averaged approximately 0.90 mS/cm more conductivity over Jerseys for each measurement taken over the five-week period.

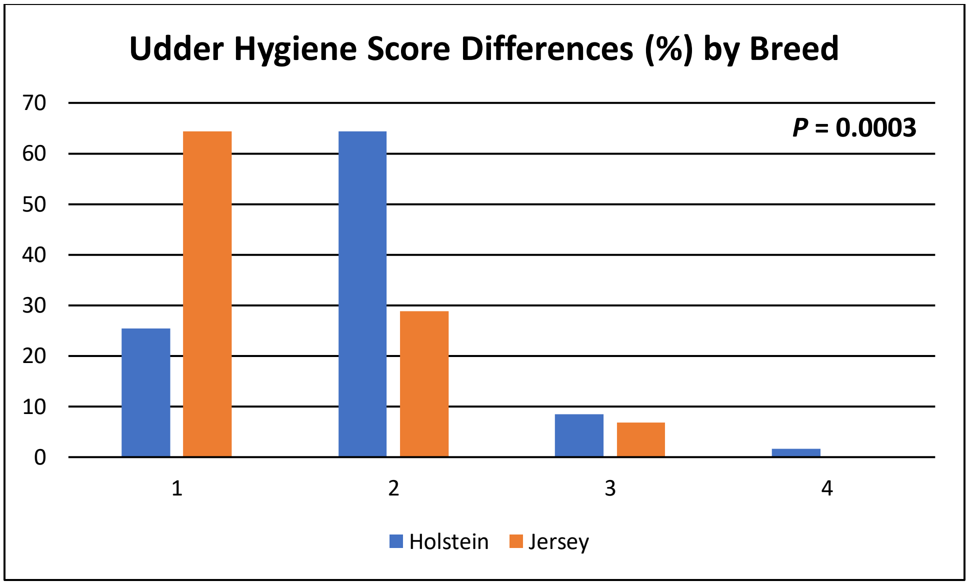


Figure 5. Differences according to breed in udder hygiene score. Jerseys were more likely to have a score of 1, whereas Holsteins were more likely to have a score of 2 or higher. It is worth noting that no Jerseys in the study scored a 4 (4 = very dirty) in udder hygiene.

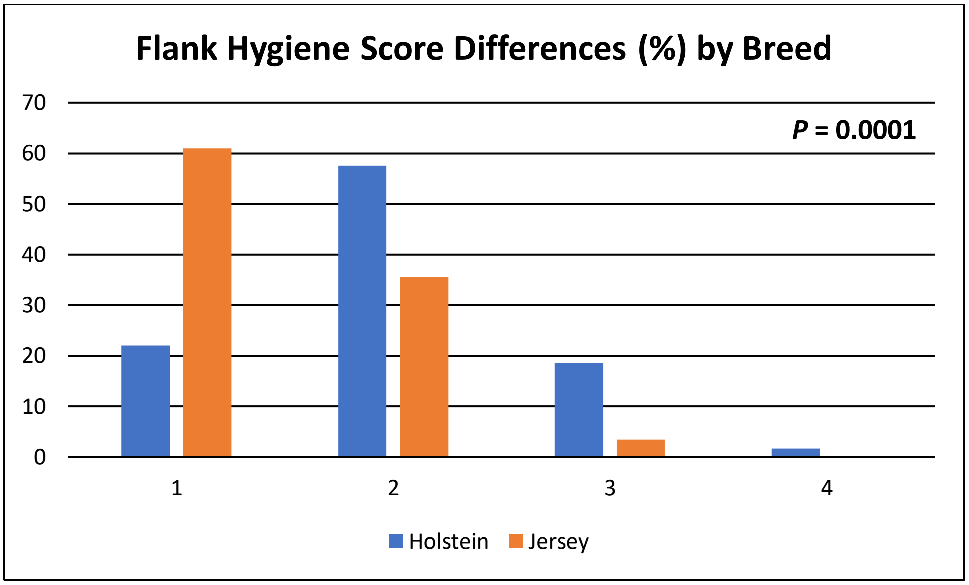


Figure 6. Differences according to breed in flank hygiene score. Jerseys were far more likely to have a score of 1, whereas Holsteins were more likely to have a score of 2 or higher. It is worth noting that no Jerseys in the study scored a 4 (4 = very dirty) in flank hygiene.

Figure 7. A hygiene scoring card which documents the degree of manure contamination on a 1-4 scale for each of three zones, the udder, the lower leg and the upper leg and flank. Score sheet available at http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf.

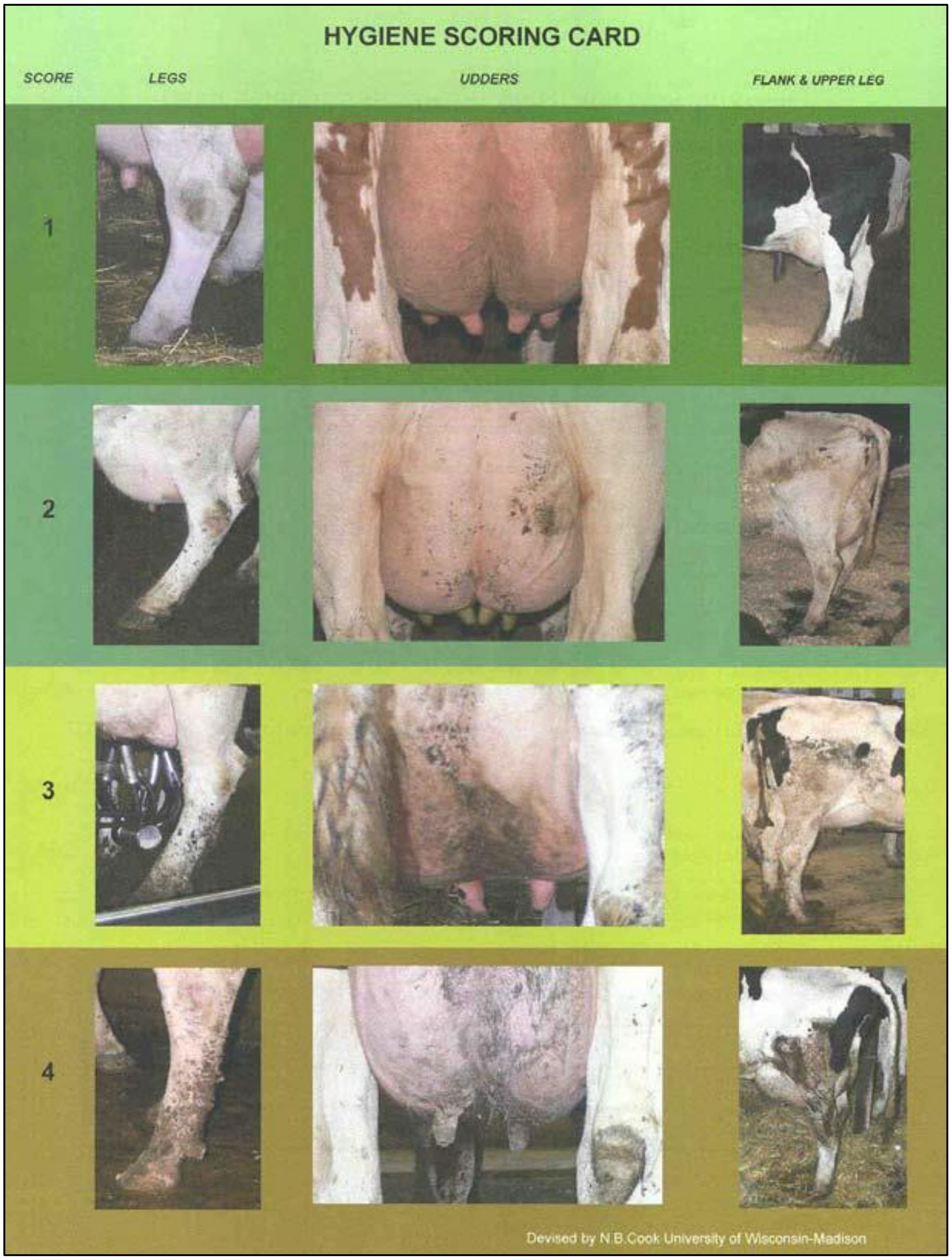
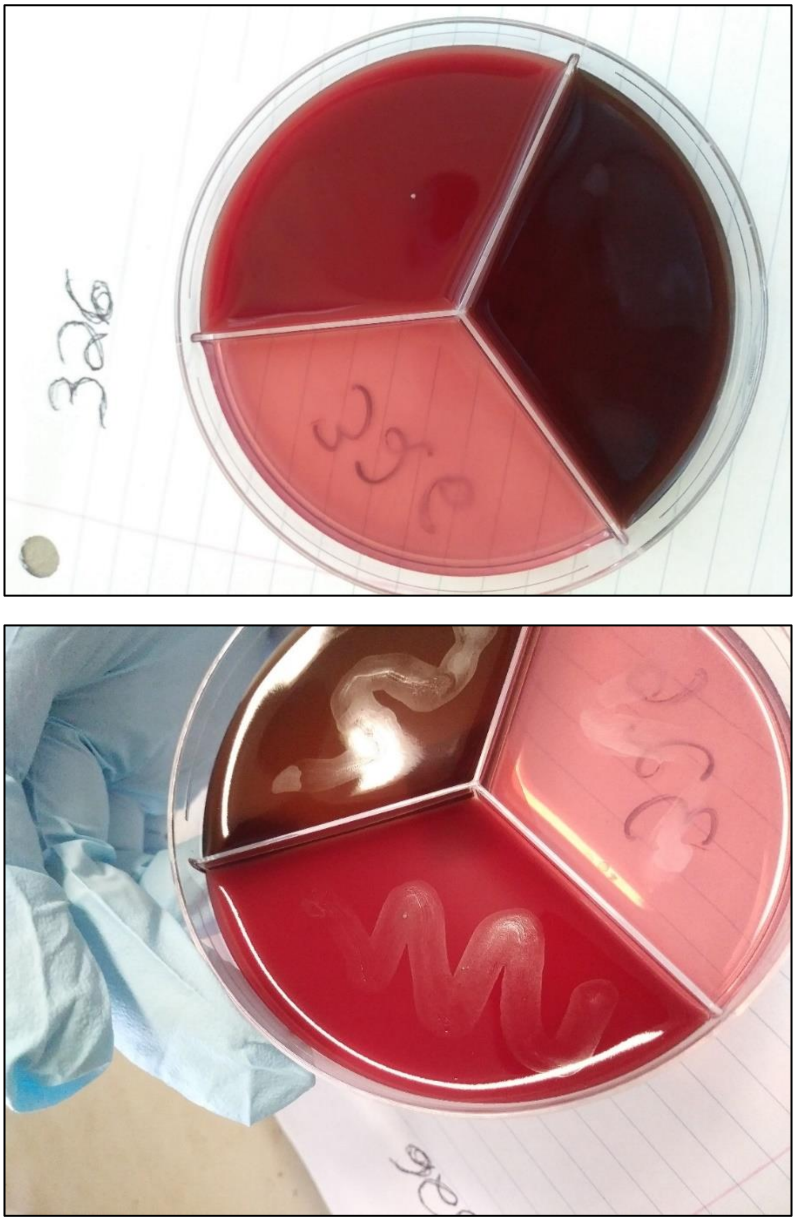
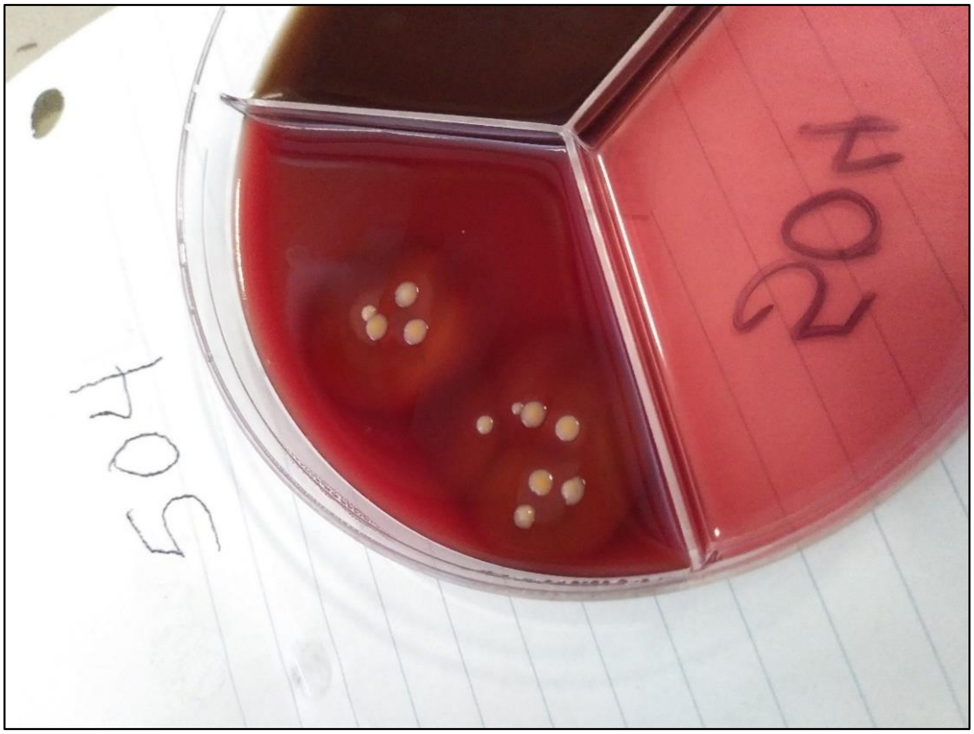


Figure 8. A tri-plate culture medium* showing "no growth." No bacteria grew after the 48-hour period.



* The deep red media is MTKT media, the scarlet media is the Factor media, and the pink media is the MacConkey media. Growth on the MTKT and Factor media indicate gram-positive bacterial growth; growth on the MacConkey media indicates gram-negative bacterial growth.

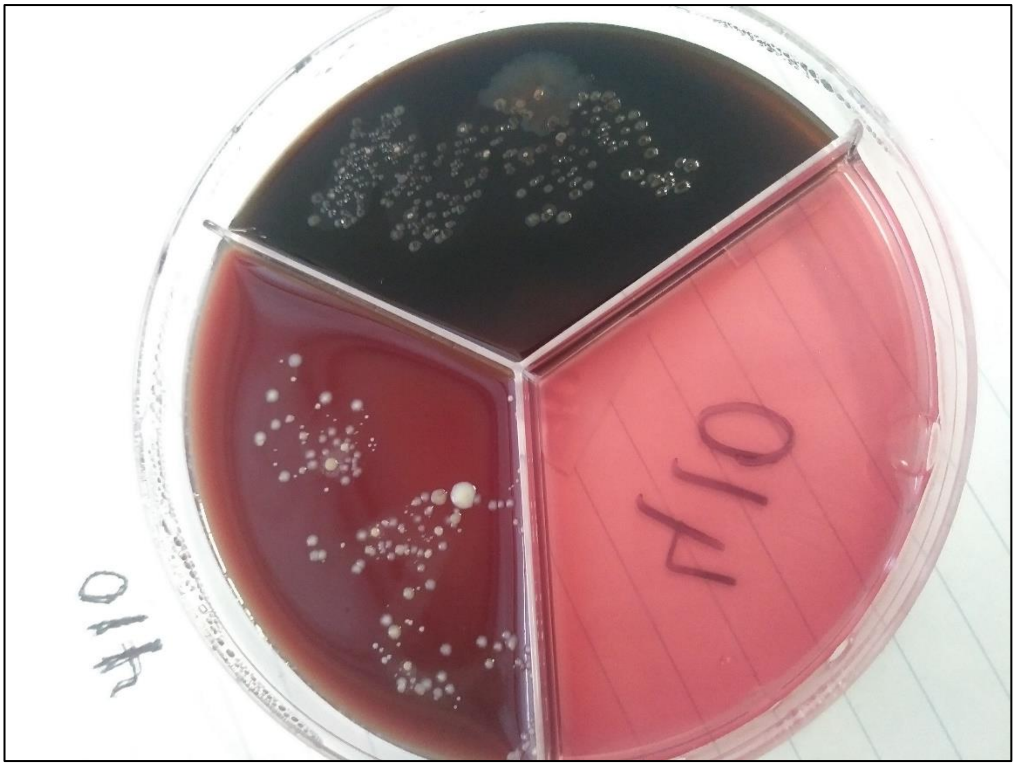
Figure 9. A tri-plate culture medium showing "*Staphylococcus aureus*" growth[a]. The zones of hemolysis around the bacteria that had cultured after 48-hours are indicative of this species.

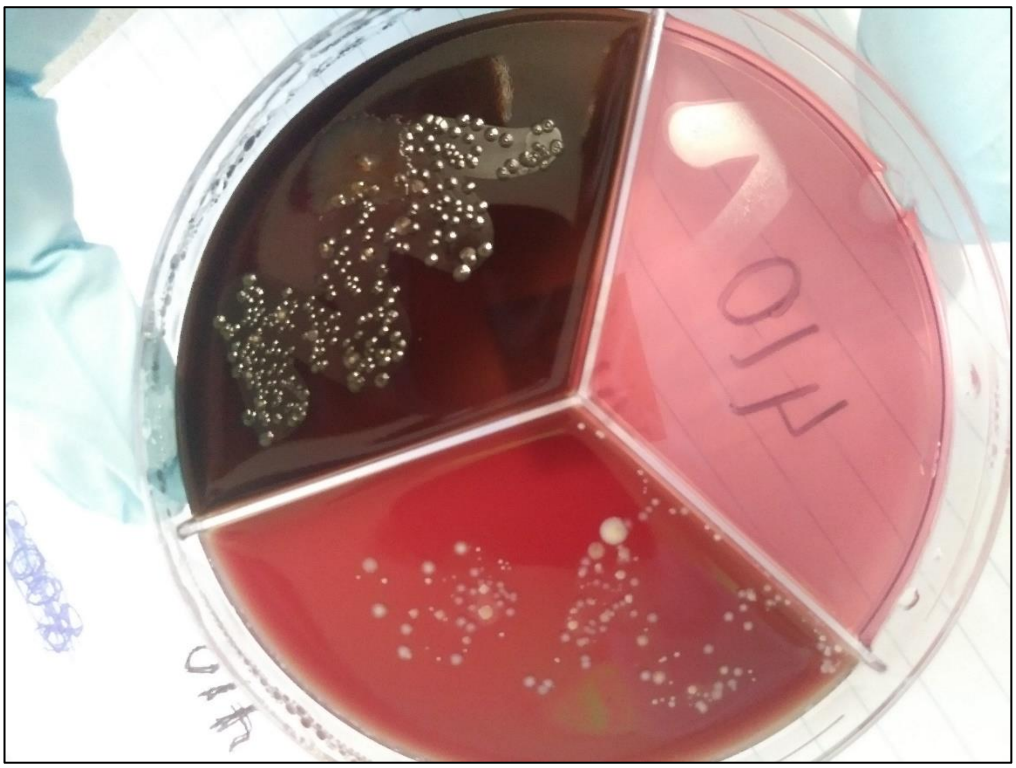


[a] *Staphylococcus aureus* growth is limited to the Factor media and is indicated by zones of hemolysis around the spots of bacteria.

Figure 10. A tri-plate culture medium showing "*Staphylococcus*" species growth (not *Staphylococcus aureus*). There are no zones of hemolysis around the bacteria that had cultured after 48-hours. Concentration in the Factor, media, however, still indicate that this is a potential *Staphylococcus* species.

Figure 11. A tri-plate culture medium showing a "*Streptococcus*" species growth[a].





[a] Concentration of the bacterial growth in the Factor and MTKT medias, with no zones of hemolysis, are indicative of *Staphylococcus aureus*.

Figure 12. The MTSU dairy parlor during evening milking.

Figure 13. Hygiene scoring in the dairy parlor as the cows are coming in to be milked.

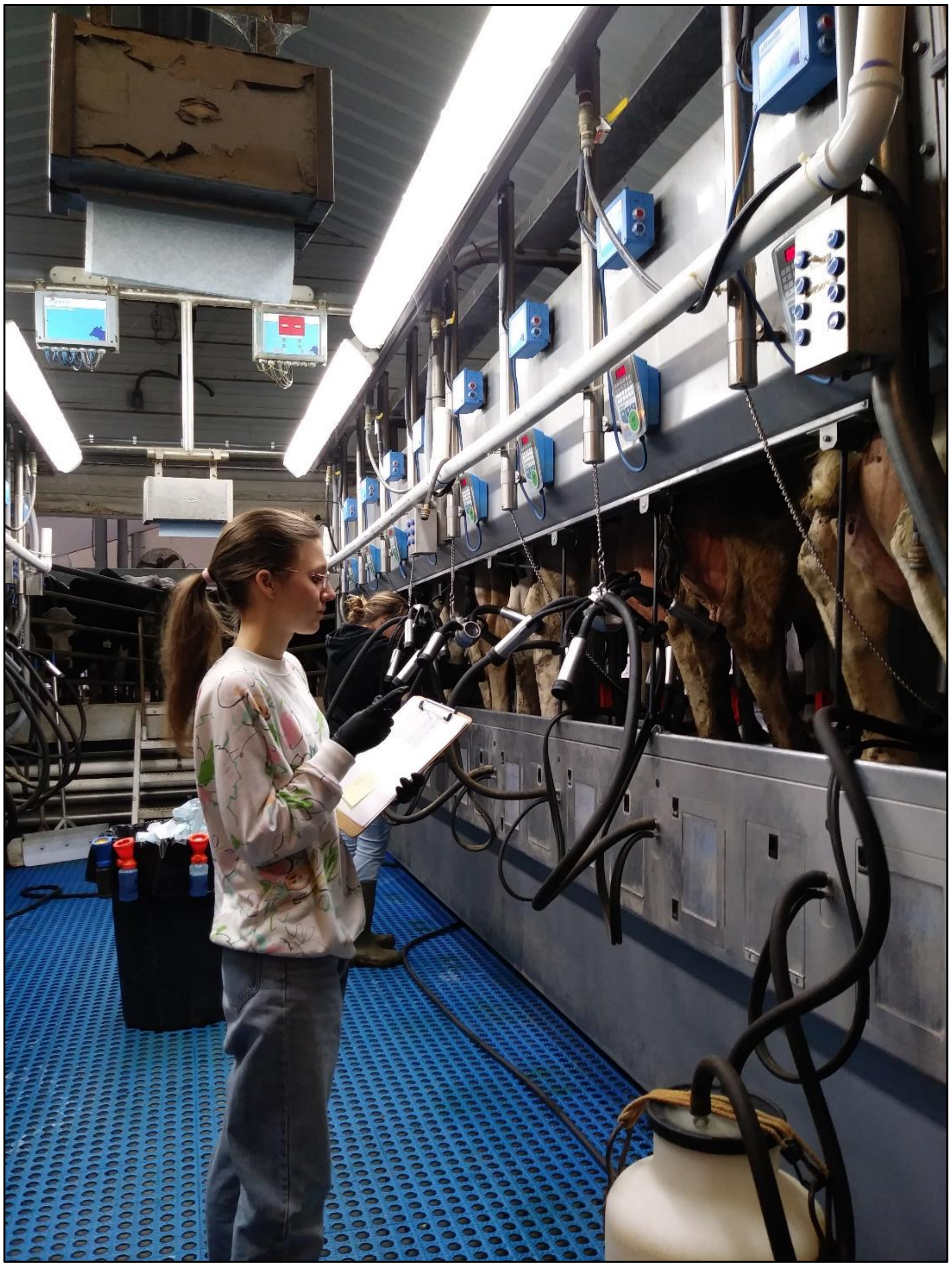


Figure 14. Hygiene scoring in the dairy parlor.

Figure 15. Taking a noncontaminated sample of milk with SCC > 350,000 cells/ml for plate swabbing.

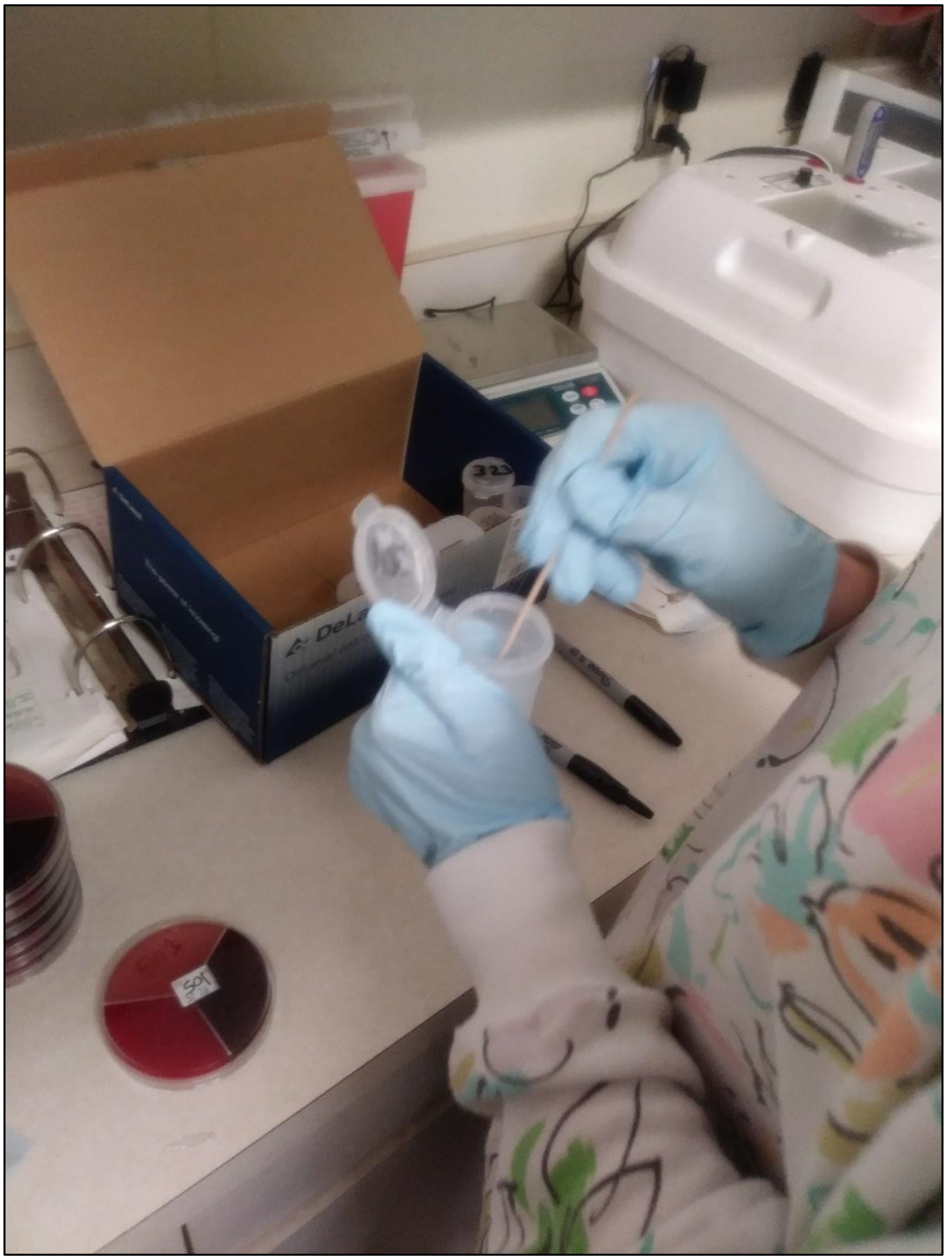


Figure 16. A tri-plate agar medium was used to culture the bacterial samples. After samples were taken, they were swabbed onto the plate to be cultured for a 48-hour period in an incubator at 98.6 degrees Fahrenheit.

Figure 17. Egg incubator wherein cultures were allowed to proliferate for a 48-hour period. The incubator was set to 98.6 degrees Fahrenheit, and cultures were checked after a 24-hour period and at the end of 48 hours.

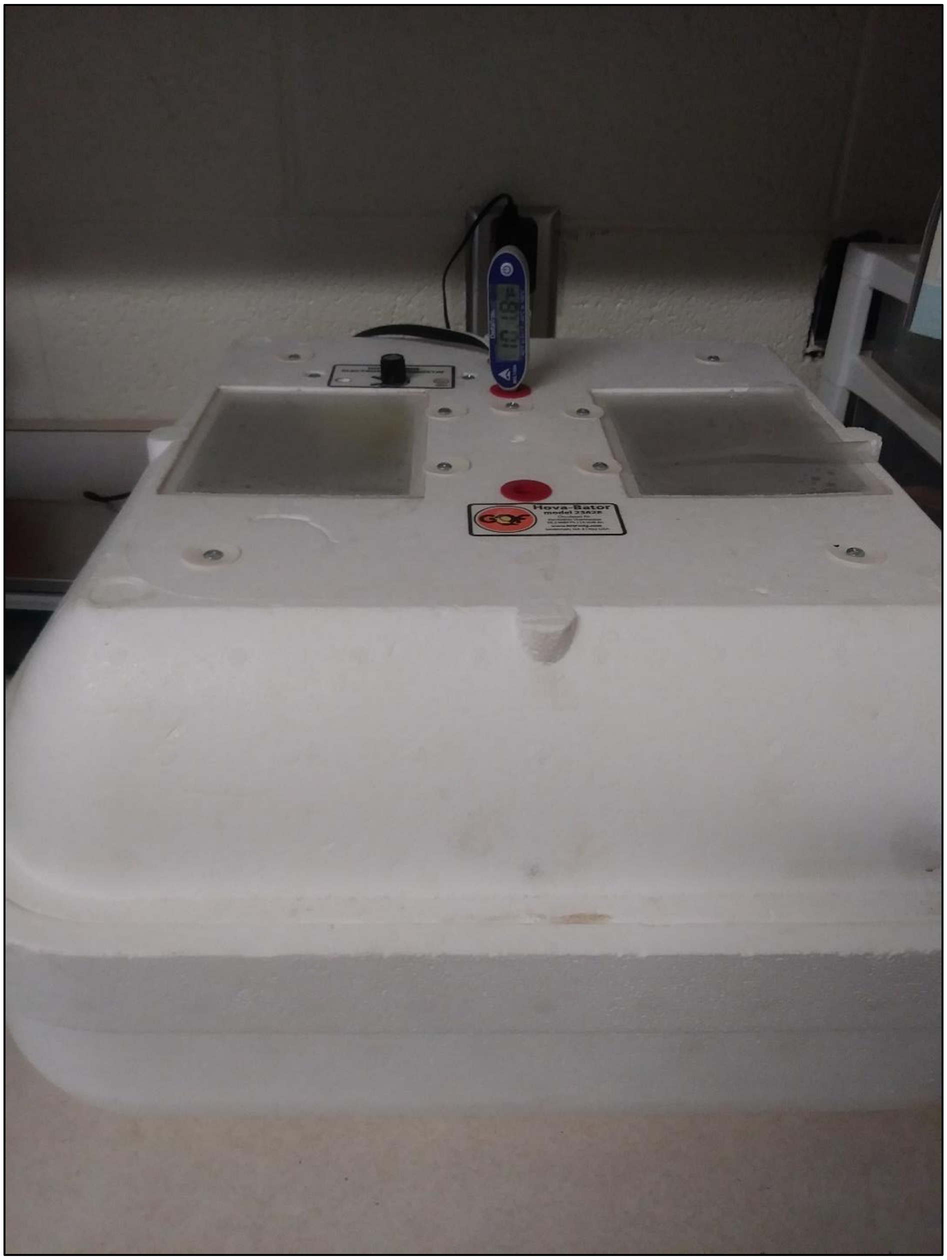


Figure 18. The DeLaval Somatic Cell Counter used to test milk samples.

Figure 19. Cartridge used to test milk samples in order to acquire an SCC reading.

IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

IACUCN001: PROTOCOL APPROVAL NOTICE

Wednesday, February 13, 2019

Senior Investigator	**Jessica Carter** (ROLE: Principal Investigator)
Co-Investigators	Britney Brown and Maegan Hollis
Investigator Email(s)	*jessica.carter@mtsu.edu; bmb7h@mtmail.mtsu.edu; megan.hollis@mtsu.edu*
Department	Agriculture
Protocol Title	***Evaluation of breed, milk production, and udder chracterisitics on somatic cell count and udder pathogens in lactating Holstein and Jersey cows***
Protocol ID	**19-2011**

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Designated Member Review (DMR) mechanism*** and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this this protocol is tabulated as below:

IACUC Action	**APPROVED for one year**
Date of Expiration	**2/28/2020**
Number of Animals	30 (THIRTY)

Approved Species	**MTSU bovine**	
Category Subclassifications	☐ **Teaching** ☐ Classroom ☐ Laboratory	☒ **Research** ☐ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: NONE	
Approved Site(s)	MTSU Dairy Farm	
Restrictions	**Satisfy DMR requirements AND annual continuing review**	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice. This protocol **expires on 2/28/2022** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved by the IACUC prior to

2/28/2020 for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule: Refer tp the following table to request your CR:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	1/31/2020	TO BE COMPLETED
Second year report	1/31/2021	TO BE COMPLETED
Final report	1/31/2022	TO BE COMPLETED

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

Post-approval Protocol Amendments:

Date	*Amendment(s)*	*IRB Comments*
NONE	NONE	NONE

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office

(On behalf of IACUC)

Middle Tennessee State University

Tel: 615 494 8918

Email: iacuc_information@mtsu.edu (for questions) and

Iacuc_submissions@mtsu.edu (for sending documents)