The Incidence of Staphylococcus aureus Mastitis in Maine Dairy Cattle

Morgan Belvin
THE INCIDENCE OF *STAPHYLOCOCCUS AUREUS* MASTITIS IN MAINE DAIRY CATTLE

by

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ABSTRACT

This project investigated the factors surrounding the incidence of *Staphylococcus aureus* (SA) mastitis in Maine dairy cattle. This type of mastitis is driven by an antibiotic resistant pathogen that is very contagious among cattle, though farmers are often unaware of how common this type of mastitis is and that it could be present in their herds. My hypothesis was that specific farm factors, like milkers performing other tasks during milking, as well as the act of pre-dipping versus not pre-dipping, would explain the increase in SA mastitis. A thirty-seven-question IRB-approved survey was subsequently distributed to farmers in the University of Maine Extension contact list and to farmer Facebook groups. Among a total of 11 responses, SA mastitis was seen in farms that do and do not pre-dip, in those whose milkers do not palpate the cow’s udder before unit removal, and in those where the cow is allowed to lie down shortly after being milked. In addition, seven of the survey respondents had a history of SA mastitis, and only two of them were able to eradicate it from their herds. Overall, the results showed that SA mastitis occurs on both small- and large-scale farms, and areas of management could be changed to decrease the presence of SA mastitis, like the frequent changing of gloves and the palpation of the udders before the milking unit is removed. This project suggested that SA mastitis is common in Maine, and farmers may be unaware of whether this pathogen is infecting their herds.
DEDICATION

I would like to thank my advisor Dr. Anne Lichtenwalner, for providing me with the opportunity to use this topic for my thesis and for her continued support throughout the completion of this project. I would also like to thank my committee members, Dr James Weber, RW Estela, Dr. David Marcincowski, and Dr. Juan Romero Gomez, for their support of me and their continued interest in this project. I would also like to thank my family; without their continued support I would not have been able to complete this journey.
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INTRODUCTION

Mastitis

One of the major concerns for a dairy herd manager is mastitis (inflammation of the udder) among cattle. Mastitis is a recurring issue in dairy herds, causing great concern for production and overall health of the herd. Known to be one of the most widespread and costly diseases among dairy cattle, when a cow comes down with mastitis, somatic cell count (SCC) increases, milk quality decreases, and performance is impaired (Kirkeby 2019). In addition, when one member of the herd comes down with mastitis, other members will likely contract the mastitis as well. Once mastitis enters the herd, several things decrease: the general health of the herd, the amount of milk production, and overall herd profitability (Berchtold 2014).

*Staphylococcus aureus* Mastitis

*Staphylococcus aureus* (SA) mastitis is a highly contagious form of mastitis that is caused by a gram-positive bacterium that is responsible for causing various forms of subclinical and clinical infections (Cheng 2020).

![Figure 1. Morphology of *Staphylococcus aureus*](https://www.ncbi.nlm.nih.gov/books/NBK441868/bin/S.aureus.jpg)

*Figure 1. Morphology of *Staphylococcus aureus***

The morphology of the SA pathogen is shown. Due to its gram-positive nature, the pathogen stains purple when viewed under a microscope. The bacteria are circular shaped and usually seen in clusters scattered throughout the slide.
This type of mastitis has been shown to result in the formation of abscesses within the milk-producing tissues of the udder, significantly reducing milk production, and decreasing the likelihood of a treatment cure (Cornell University). This pathogen can be transmitted to the cow through the environment or the milking systems, as well as via fomites like the hands of milkers (Jamali 2014). Due to its subclinical and asymptomatic characteristics, this infection is difficult for farmers to detect, leading to an increase in the rate of transmission among the herd (Cheng 2020). Being gram-positive, this bacterium has a thick peptidoglycan layer, making it highly absorbable, and easier to kill than gram-negative bacteria, because of its lack of an additional cell membrane (Brennan 2021).

However, SA bacteria are very tolerant to extreme changes in temperature, and can be resistant to various antibiotics, making them very difficult to kill (Malik, 2019).

**Antibiotic Resistance**

Due to the practice of incorporating antibiotics into bovine feed to treat and control diseases, antibiotic resistance is becoming an issue, as pathogens are developing resistance to these antibiotics due to over-administration (Jamali 2014). In previous studies, SA bacteria have been shown to possess a variety of antibiotic resistance genes such as *meca* (conferring resistance to Oxacillin), *tetM* (Tetracycline), *ermA* (Erythromycin), *blaZ* (Penicillin), *aacA-aphD* (Gentamicin), *ant(4’-Ia)* (Kanamycin and Tobramycin), and *fexA* (Chloramphenicol) (Jamali 2014). Research suggests that due to these antibiotic resistance genes, SA bacteria are approximately 100% resistant to antibiotics like Kanamycin and Oxacillin, approximately 92% resistant to Augmentin and
Cefoxitin, and approximately 84% resistant to Ampicillin and Erythromycin (Malik 2019).

Infections of SA stem from the expression of virulence factors. Many virulence factors have been identified from isolates of SA mastitis, including toxic shock syndrome toxin-1 (TSST-1), enterotoxins, enterotoxin-like exfoliative toxin, as well as Panton-Valentine leucocidin. These virulence factors may be highly resistant to heat as well as to proteolytic enzymes (enzymes that break down protein), which enable the bacteria to survive and multiply in the mammary gland, adding to the factors that make the bacteria difficult to kill (Liu 2017).

**Risk for Infection to Humans**

With SA bacteria, it is extremely important to understand that the SA pathogen is zoonotic and can be transferred to humans. Once transferred to humans, this pathogen can cause a variety of severe life-threatening infections of the skin, bloodstream, respiratory system, and the urinary system (Feingold 2012). Due to its antimicrobial resistance, this pathogen, if found in a hospital, has the potential to spread rapidly and cause a significant number of infections. In addition, once infected, humans can asymptptomatically carry this infection on their skin or in their noses, leading to a greater risk of transmission and the potential for an outbreak.

The specific strain of SA known as MRSA (Methicillin Resistant *Staphylococcus aureus*) is a significant cause of nosocomial (originating in a hospital) infections in humans across the world (Jamali 2014). This strain of SA is usually highly resistant to many antibiotics and very difficult to treat. This infection is usually spread from skin-to-
skin contact, and once in the body, can spread to the bones, joints, blood or any major organ like the heart and brain. If the infection does not spread beyond a wound on the skin, it can be treated by draining the respective infected area. However, once the infection has spread beyond the skin, it is extremely difficult to treat and becomes dangerous for the individual infected (Penn Medicine).

In 2014, a study was conducted in Iran, attempting to identify the prevalence of antibiotic resistance of SA isolated from bovine clinical mastitis. The results of the study showed that of the 207 infected milk samples collected, 43 (or 20.7%) of the samples were identified as SA, and of that 20.7%, 5 (or 8.6%) of those samples were identified as being Methicillin Resistant (MRSA) (Jamali 2014). This study shows that cattle can carry MRSA infections in the form of mastitis, further depicting the possibility of zoonotic transfer.

In addition to cattle transferring this pathogen to people, humans can also transfer this pathogen to cattle. Humans who have this infection can carry it through their mouths and noses, and if sanitation measures are lacking, a milker can transfer the infection to a cow, simply by wiping their nose with their gloved hands and then proceeding to milk the cow.

Potential Factors Increasing Risk of SA Infection

Intramammary infections like SA mastitis have been shown to be linked to subclinical infections that result in reduced milk quality and production. SA intramammary infections are caused by this contagious pathogen that transfers from cow to cow during milking via contaminated milking equipment and the hands of milkers (Svennesen 2019). Therefore, some research has suggested that the teat skin might serve
as a reservoir for contagious pathogens to enter the udder through the teat canal and cause these intramammary infections. SA bacteria can be isolated from the teat skin, and teat skin colonization with SA has been epidemiologically associated with SA intramammary infections (Svennesen 2019).

A 2019 study was conducted to identify the probability of developing SA mastitis due to teat skin colonization with SA, and the use of automatic milking systems. When using automatic milking systems, the sources of SA bacteria are not the hands of milkers, but rather the challenge to hygiene due to the standard teat cleaning process before and after milking (Dohmen 2010). Of 1,142 skin and milk samples collected from quarters of 300 cows, SA was found in 93 (or 8.1%) of the milk samples, 75 (or 6.6%) of the teat skin samples, and of those samples, 15 (or 1.3%) of the quarters were positive for SA in both the teat skin and milk samples. In addition, after a multivariable analysis was performed, the results showed that quarters positive for SA skin colonization were 7.8 times more likely to have SA mastitis, versus those negative for SA colonization on the teat skin (Svenneesen 2019). This study demonstrates that SA skin colonization greatly increases the chances of developing SA mastitis, especially in automatic milking systems.

Due to the severity of this pathogen and the great economic loss that comes with SA infections, discovering what farm-based factors are causing this type of mastitis to occur on dairy farms is important. Some research has suggested that a cow’s stage of lactation, as well as teat-end roughness and callosity, increases the incidences of SA mastitis. In addition, those cows who have had a previous SA mastitis infection that farmers were able to get rid of, are also more prone to developing this type of mastitis again (Zadoks 2001). In terms of management practices, studies have shown that factors
like overmilking, undermilking, the time lapse between pre/post dipping and the placing/removal of the milking unit, the lack of good sanitation practices, as well as the lack of isolation of new pregnant heifers into the herd, have caused SA mastitis (Malik 2019).

In terms of prevention, some studies have suggested that by following milking practices like prestrip, pre- and post-dip, and use of a single towel per cow to dry teats, a farmer can decrease presence of SA mastitis on their farms. In addition, data has also supported the idea that segregating SA infected cows away from healthy cows, as well as milking SA-infected cows last, will decrease a farmer’s chances of contracting SA mastitis. Farmers taking an active role, such as supervising their hired employees while milking, also may experience a decrease in SA mastitis, suggesting that regular training and motivation of employees can be a beneficial way of preventing SA mastitis (Costa 2016).

**Treatment of SA Mastitis**

Due to the severity of this type of mastitis, treatment varies based on a variety of conditions. The probability or odds of curing the cow depends on the cow itself, the strain of the pathogen and the treatment factors. The chances of curing the cow decrease with the increasing age of the cow, increasing SCC, increasing duration of infection, increasing bacterial colony counts, as well as increasing number of quarters infected on the cow (Barkema 2006). SA mastitis in hind quarters is more difficult to treat than that of SA mastitis in front quarters. However, the most effective treatment factor is the duration of treatment. The longer the farmer tries to get rid of the SA mastitis, the greater the chances of cure (Barkema 2006).
Objectives

The goals of this study were to investigate specific farm-based factors that might lead to an increase in SA mastitis, and after identifying these factors, to give recommendations to farmers on how to manage SA mastitis on their farms.

Research Question

Which farm-based factors--such as the time lapse between taking off the milking unit and post dipping, or the type of flooring in the barn--lead to an increase in SA mastitis?

Hypothesis

It was hypothesized that specific farm factors, like milkers performing other tasks during milking, as well as the act of pre-dipping vs not pre dipping, would explain the increase in SA mastitis
MATERIALS AND METHODS

Survey Creation

A survey was created to gain information about the management practices of various dairy farmers across Maine. This survey asked farmers about their milking practices, as well as their farm management practices. The survey was created on Qualtrics and had thirty-seven questions that consisted of mostly multiple-choice with a few open-ended questions.

Survey Approval

Prior to distribution of the survey, both the informed consent and questions were sent to the University of Maine Institutional Review Board (IRB) for approval. The survey was deemed “non-human subjects” related (exempt from full IRB approval). The study adhered to the Office of Research Compliance guidelines for confidentiality and transparency, as requested by the IRB.

Survey Distribution

The survey was first sent out to the members of the dairy contact list by courtesy of Donna Coffin in the University of Maine Cooperative Extension. After receiving very few responses through this extension list, the survey was shared with Maine farmer groups on Facebook.
RESULTS

Survey Responses

A total of eleven responses were received, with one response only having a quarter of the questions answered. Ten out of eleven respondents were dairy farmers from the state of Maine, and one respondent was a dairy farmer from the state of Kansas. The lack of survey responses will be accounted for in the discussion portion of this Thesis.

Demographics

Out of eleven respondents, approximately 82% of the farmers had been dairy farming for greater than five years. The remaining 18.2% of respondents had been farming between 2-5 years. Most of the respondents were non-organic farms, with only 36% of the respondents practicing organic farming. Approximately 46% of the respondents were milking Holstein cattle, followed by 27% milking Jersey cattle, 9% with Milking Shorthorn cattle, 9% with Guernsey cattle, and 9% with a combination of the above.
A majority of the farms had fewer than 50 cows in production simultaneously.

Approximately 36% had greater than 100, and the remaining 9% had between 50-100 cows in production simultaneously.
Figure 3: Type of Milking System

Most respondents used an elevator parlor with a milk line system. However, SA mastitis was seen in all types of milking systems except manual/labor milking, suggesting that milker attention to detail during milking could be a factor leading to the presence or lack of SA mastitis.
Figure 4: Relationship Between Pre-dipping and the Presence of SA Mastitis.

Out of 11 farms, SA mastitis was seen in both those that do and do not pre-dip, but proportionately more common in those that pre-dip. Suggesting that the act of pe-dipping might not be a factor in SA mastitis.
Figure 5: Type of Dipping System

Most of the respondents stated that they use an ambic non-return teat dipping system, and most of them use chlorohexidine as pre-dip, and place the unit on approximately 1-2 minutes after pre-dipping. SA Mastitis was seen in users of all the above dipping systems except those that used moistened towelettes as a wipeout practice. Suggesting that the use of towelettes instead of dip cups could be a positive factor in the decrease of SA mastitis.
Figure 6: Palpation of Udders

Most of the farms palpate the udders before the removal of the unit. By palpating the udder, milkers can feel if milk is still left in the cow before the unit is taken off. However, a large proportion of farms who do not palpate, and residual milk could still be present in the cow, leading to undermilking and high risks of contracting SA mastitis.
Figure 7: Duration of Time Post Milking

Giving the teat canal time to close after milking decreases risk of infection.

About 1/3 of the farmers allow the cow to lie down within less than 5 minutes after milking, which increases the risk of udder infection and suggests that areas of management could be improved.
All the respondents stated that their milkers change their gloves after every milking. In addition, 50% of the farms’ milkers do not sanitize their gloved hands between cows, enabling the transfer of bacteria like SA between cows during milking.
Figure 9: Mastitis Cows

Approximately 70% of the farms milk the mastitis cows after all non-infected cows. However, approximately 60% of the farms do not house their mastitis cows separately from infected cows, and approximately 10% house them in the sick pen, which could expose the cow to more potential health issues, like respiratory disease.
SA mastitis was seen in users of all bedding options, with most respondents using wood products, suggesting that bedding might not be correlated with SA mastitis.
Figure 11: Frequency of Veterinarian Visitation

About 2/3 of the respondents have a regular veterinarian who does a herd check every 6 months. A small proportion have a veterinarian visit only when a cow is displaying signs of illness or mastitis.
Figure 12: Vaccination Against SA Mastitis

Most of the farmers do not vaccinate against SA mastitis and approximately 60% of them who had it in their herds were never able to eradicate it.
Out of 11 farms, 70% have had a history of SA mastitis, with only 2 farms never having SA mastitis, 1 farmer unsure, and 1 blank response. This demonstrates how common SA mastitis is, and the need to narrow down what is causing it to happen on dairy farms.
Table 1. Use of DHIA Services, Production Goals and SA Mastitis

<table>
<thead>
<tr>
<th>Respondents</th>
<th>Utilize DHIA services?</th>
<th>Sets production goals?</th>
<th>Meets production goals?</th>
<th>Case of SA mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>Yes</td>
<td>Yes</td>
<td>Not sure</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between the use of DHIA milk testing services, the use of production goals, and the incidence of SA mastitis. Out of eleven respondents, nine of the respondents, or approximately 81% stated that they use DHIA milk testing services, and of those nine respondents, approximately 77% set production goals, and of that 77%, approximately 42% stated that they meet their production goals. However, SA mastitis was seen in those that do and do not use DHIA services, as well as those that do and do not set and meet herd production goals.
Table 2. Quarantine and Outside Exposure Measures and SA Mastitis

<table>
<thead>
<tr>
<th>Respondents</th>
<th>Community Pastures?</th>
<th>Quarantine of New Pregnant Heifers?</th>
<th>Case of SA Mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>No</td>
<td>Yes</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between the quarantine of new pregnant heifers and the use of community pastures and the occurrence of SA mastitis. Only one respondent stated that they do not send their cows to community pastures, and they do quarantine new pregnant heifers, and they have never had a case of SA mastitis. The only other respondent that did not have a case of SA mastitis does not quarantine new heifers and sends their cows to community pastures. The remaining respondents all had SA mastitis, with two respondents not knowing if they ever had it, and these responses were mixed between those that do and do not send their cows to community pastures and those that do and do not quarantine new pregnant heifers. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
Table 3. Type of Barn Flooring and SA Mastitis

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Type of Flooring</th>
<th>Case of SA Mastitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>Concrete</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>Gravel</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>Concrete</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>Brick</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>Brick</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between the type of flooring in the dairy barn and the presence of SA mastitis. Approximately 63% of the respondents have concrete flooring in their barn, and all those respondents have had a case of SA mastitis. Only one respondent had gravel flooring in their barn, and they also had a case of mastitis. However, the two respondents that had brick flooring in their barns have never had a case of SA mastitis, demonstrating that this type of flooring could be a positive factor in the decrease of the incidence of SA mastitis. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
Table 4. Number of Cows and Milkers per Session and SA Mastitis

<table>
<thead>
<tr>
<th>Respondents</th>
<th>Number of cows being milked per milking session</th>
<th>Number of milkers per milking session</th>
<th>Case of SA mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>2</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>10</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>16</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>4</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>16</td>
<td>1-2</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>N/A</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>8</td>
<td>1</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>40</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>6</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>4</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>25</td>
<td>1</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between the number of cows being milked per session, the number of milkers, and the occurrence of SA mastitis. The two respondents who did not have a case of SA mastitis are milking a small number of cows, and only have one and two milkers. The remaining farms who have had SA mastitis are also milking a small number of cows with either one or two milkers; suggesting that the occurrence of SA mastitis must have more to do with the milkers, and the degree to which they are paying attention to the cows during the milking. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
Table 5. Type of Post-dip and SA Mastitis

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Post-dip?</th>
<th>Type of Post-dip?</th>
<th>Case of SA Mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>Yes</td>
<td>Iodine</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>Yes</td>
<td>Iodine</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>Yes</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>No</td>
<td>N/A</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>Yes</td>
<td>Chlorhexidine</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>Yes</td>
<td>Iodine</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>Yes</td>
<td>Iodine</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>Yes</td>
<td>Iodine</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>Yes</td>
<td>Sodium Hypochlorite</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>Yes</td>
<td>Iodine</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>No</td>
<td>N/A</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between whether the farmer post-dips, what type of post-dip they use, and whether they had a case of SA mastitis. Only two of the respondents did not have SA mastitis, with one who does not post-dip their cows, and one who does post-dip and uses sodium hypochlorite. The remaining respondents all had SA mastitis, with 85% of them using iodine as their post-dip, and one not post-dipping. This suggests that the use of iodine as a post-dip solution might not be a positive factor in the decrease of SA mastitis. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
Table 6. Milkers Working While Sick and SA Mastitis

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Do your milkers still milk when they are ill?</th>
<th>Case of SA Mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>Yes</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table show the correlation between whether a milker is allowed to milk while ill, and the incidence of SA mastitis. All the respondents who had a case of SA mastitis were mixed with some stating that they do allow their milkers to still milk when they are ill, and some that do not. However, the two farms that did not have a case of mastitis stated that their milkers do not milk while ill, suggesting some cases of SA mastitis could be coming from infected milkers. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
Table 7. Other Activities During Milking and SA Mastitis

<table>
<thead>
<tr>
<th>Respondent</th>
<th>Do your milkers Perform Other Tasks During Milking?</th>
<th>Case of SA Mastitis?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respondent 1</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 2</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 3</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 4</td>
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<td>Yes</td>
</tr>
<tr>
<td>Respondent 5</td>
<td>No</td>
<td>Not sure</td>
</tr>
<tr>
<td>Respondent 6</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 7</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 8</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Respondent 9</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Respondent 10</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Respondent 11</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

The above table shows the correlation between whether the milkers perform other barn related tasks while milking and if the farmer had a case of SA mastitis. Surprisingly, the two respondents who did not have a case of SA mastitis do have their milkers perform other barn tasks while milking. The remaining respondents who had a case of SA mastitis, were a mix between those that do and do not have their milkers perform other tasks while milking, demonstrating this may not necessarily be a factor in the increase of SA mastitis. Respondent 5 was not sure if they had SA mastitis, and Respondent 10 did not answer the question about their history of SA mastitis.
DISCUSSION

Demographics

The farm respondents in this survey differed greatly in terms of facility size, milking systems, and management practices, but SA mastitis was seen in all types of farms. Most farmers are practicing effective safety measures, but SA mastitis is still being found on their farms.

Management Practices

In terms of the management styles being used on these dairy farms, the results of this study suggest that some areas could be improved to attempt to decrease the incidence of SA mastitis on these farms.

While the survey result yield was very low, it provided some initial information for more studies to be done. For example, two of the respondents who did not have a case of SA mastitis stated that their dairy barn has brick flooring instead of concrete or gravel. This suggests that brick flooring could be a positive contributor in the decrease of SA mastitis, but more data is needed to validate this piece of information.

In this study, all the farms stated that they only change their gloves after every milking, and most of them do not sanitize their gloves hands in between cows. To decrease the incidence of SA mastitis on these farms, farmers need to implement more sanitary measures during milking. The simple act of changing gloves every few cows or sanitizing gloved hands in between cows can prevent bacteria from being transferred from one cow to another; this will decrease the likelihood of SA mastitis infecting all the cows in the herd.
In addition to the sanitization of gloved hands or the changing of gloves in between cows, the act of palpating a cow’s udder before removing the milking unit is extremely important. A milker cannot solely rely on the amount of milk present in the claw of the unit to make their decision of whether the cow is milked out. The lack of palpating udders can lead to the cow being undermilked because residual milk is still left in their udders. By palpating the udders, the milkers will be able to feel whether the cow has milk left or if the unit can be removed, decreasing the chances of undermilking occurring. In addition, constantly palpating the cow’s udder while they are milking out, will also decrease the chances of the cow being overmilked. A decrease in the instances of both undermilking and overmilking will decrease the chances of the cow contracting SA mastitis.

In addition to the palpation of udders, increasing the amount of time that a cow must stand before lying down is extremely important in preventing the incidence of SA mastitis. When a cow is being milked, the teats are open and exposed to the atmosphere, also demonstrating the importance of pre-dipping and post-dipping. However, even after post-dipping, it takes time for the teat canal to completely close. Approximately 1/3 of the respondents in this survey allow their cows to lie down less than five minutes after the unit is removed; this increases the chances of the cow contracting bacteria like SA, because their teats are still open, and are exposed to any pathogens living in the bedding.

The placement and order to which the mastitis cows are milked on a dairy farm is extremely important when managing the rate of infection and decreasing the chances of transmission. All mastitis cows should be milked after all the non-infected cows to avoid the bacteria being transferred to healthy cows. In addition, the infected cows should be
housed separately from the healthy cows. However, approximately 60% of the respondents do not house the mastitis cows separately from the healthy cows, and approximately 10% of those respondents house the mastitis cows in the sick pen. This should be avoided, because if the cow already has mastitis, their immune systems are compromised, and they are at risk of easily contracting another sickness.

In terms of the use of SA vaccines, most of the farmers do not vaccinate against SA mastitis, and approximately 60% of those who have had SA mastitis were not able to eradicate it from their herds. There are many mixed reviews about the effectiveness of this vaccine, and many farmers choose not to use it. However, data has shown that the SA vaccine can be effective, in combination with effective management practices and safety measures. Administering the vaccine while also sanitizing gloved hands and palpating udders can help to decrease a farmer’s chance of contracting SA mastitis.

These survey results also provided some surprising information, as SA mastitis was seen more in farmers whose milkers do not perform other tasks during milking, than those who do not, demonstrating the need for more research with a higher yield of results to be done. In addition, SA mastitis was seen in both farms that do and do not quarantine their new pregnant heifers prior to calving, and those who do and do not send their cows to community pastures. However, for both factors, there was not a noticeable enough difference to make a distinction of whether they are specifically causing SA mastitis due to the lack of results.

In terms of veterinarian visitation, approximately 2/3 of the respondents have a bovine veterinarian routinely visit their herds every six months, while a small portion only have a veterinarian come when a cow is sick. By having a veterinarian routinely
visit their herds, farmers can constantly analyze their management practices with the advice of their veterinarian, which could lead to the decrease in SA mastitis. However, due to the lack of results, a concrete connection cannot be made between the frequency of veterinarian visitation and the presence of SA mastitis.

The main take-away from this project is the need for farmers to be mindful and proactive about their milking and management practices. The results obtained from this survey are suggesting that most of the factors leading to the incidence of SA mastitis are milker related. This survey showed that it is extremely important for farmers to implement strict milking procedures and specific instructions for their milkers. In addition, it is important for farmers to observe their milkers for a period after initial milking training. By observing their milkers, farmers can observe the actions of their milkers and address any issues or concerns prior to infection occurring.
CONCLUSION

The goals of this project were achieved as the results suggested that certain farm-based factors are leading to the increase of SA mastitis on Maine dairy farms. This project suggested that SA mastitis is very common in Maine, and many farmers may be unaware whether it is present in their herds, due to the subclinical nature of the pathogen. More studies should be done to further narrow down what specific factors are causing this to occur to help dairy farmers keep this type of infection away from their farms.
REFERENCES


APPENDIX A: IRB APPROVAL: NON-HUMAN SUBJECTS RELATED

APPLICATION COVER PAGE

- KEEP THIS PAGE AS ONE PAGE – DO NOT CHANGE MARGINS/FONTS!!!!!!!!!
- PLEASE SUBMIT THIS PAGE AS WORD DOCUMENT

APPLICATION FOR APPROVAL OF RESEARCH WITH HUMAN SUBJECTS
Protection of Human Subjects Review Board, 311 Alumni Hall

(Type inside gray areas)

PRINCIPAL INVESTIGATOR: Morgan Belvin EMAIL: morgan.belvin@maine.edu
CO-INVESTIGATOR:EMAIL:
CO-INVESTIGATOR:EMAIL:
FACULTY SPONSOR: Dr. Anne Lichtenwalner EMAIL: anne.lichtenwalner@maine.edu
(Required if PI is a student):

TITLE OF PROJECT: The Prevalence of Staph aureus mastitis in bovine dairy farms across Maine
START DATE: 10/18/2021
PI DEPARTMENT: AVS
STATUS OF PI: FACULTY/STAFF/GRADUATE/UNDERGRADUATE Undergraduate (F,S,G,U)

If PI is a student, is this research to be performed:

☐ for an honors thesis/senior thesis/capstone? ☐ for a master’s thesis?
☐ for a doctoral dissertation? ☐ for a course project?
☐ other (specify)

Submitting the application indicates the principal investigator’s agreement to abide by the responsibilities outlined in Section I.E. of the Policies and Procedures for the Protection of Human Subjects.

Faculty Sponsors are responsible for ensuring that he/she has read the application, agrees with the aims of the research, and ensures that the research is conducted in accordance with the University of Maine’s Policies and Procedures for the Protection of Human Subjects. REMINDER: if the principal investigator is an undergraduate student, the Faculty Sponsor MUST submit the application to the IRB.

Email this cover page and complete application to umir@maine.edu.

******************************************************************************
FOR IRB USE ONLY Application # 2021_10_02 Review (F/E): Expedited Category:

ACTION TAKEN:
☐ Judged Exempt; category Modifications required? Accepted (date)
☐ Approved as submitted. Date of next review: by Degree of Risk:
☐ Approved pending modifications. Date of next review: by Degree of Risk:
☐ Modifications accepted (date):
☐ Not approved (see attached statement)
☒ Judged not research with human subjects

FINAL APPROVAL TO BEGIN 10/18/2021
Date 10/2018

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APPENDIX B: INFORMED CONSENT

You are invited to participate in a research study conducted by an undergraduate student at the University of Maine. My name is Morgan Belvin, and I am a student in the Animal Veterinary Sciences department in the college of natural sciences, forestry, and agriculture at the University of Maine. For my senior project, I will be researching the presence of Staph aureus mastitis in dairy farms across Maine. I will be working alongside Dr. Anne Lichtenwalner, (an associate professor and extension veterinarian,) to analyze milk data over the span of the last ten years to determine the presence of SA mastitis. I invite you to participate in this survey as a part of my research project to help me determine what farm-based factors contribute to the presence of SA mastitis. To participate in this survey, you must own or have owned a dairy farm and must be eighteen years of age or older.

Please click on the link below to learn more about the study and take the survey

**What will you be asked to do?**
You will be asked to participate in an anonymous survey that asks about your farm’s management practices and styles. This survey will take approximately five to ten minutes to complete.

**Risks**
Except for your time and inconvenience, there are no risks to you from participating in this study.

**Benefits**
The overall benefit of the project will be to come up with a management plan to help farmers get rid of SA mastitis on their farms. Your participation in this project will allow me to attempt to isolate what is causing the presence of SA mastitis and come up with different ways for farms across Maine to implement new styles of management to eliminate their farms of this disease.

**Confidentiality**
All participants in this survey will remain anonymous. No IP addresses will be collected, and no identifiable information will be collected. There will be one personal question that asks how long the farmer has been in this business, but that will be optional and may be skipped. Any work that is published from this data will not include any identifying information. The survey data will be saved on a password protected computer and will be kept indefinitely.

**Voluntary**
Your participation in this study is voluntary

**Contact information**
If you have any questions or concerns about this survey, please contact myself, Morgan Belvin at morgan.belvin@maine.edu or Dr Anne Lichtenwalner at anne.lichtenwalner@maine.edu.

*By participating in this survey, you understand the above information and consent to participate in this survey*
APPENDIX C: QUALTRICS SURVEY QUESTIONS

Q1 How long have you been farming?
   o 1 year (1)
   o 2 to 5 years (2)
   o Greater than 5 years (3)

Q2 Do you practice organic farming?
   o No (1)
   o Yes (2)

Q3 What breeds of dairy cattle do you have on your farm?
   o Holsteins (1)
   o Jerseys (2)
   o Brown Swiss (3)
   o Ayrshires 4)
   o Milking Shorthorns (5)
   o Combination (please specify which combination) (6)

Q4 On average, how many of your cows are in production (lactating) simultaneously?
   o Fewer than 50 (1)
   o Between 50-100 (2)
   o Greater than 100 (3)

Q5 Do you utilize DHIA milk testing and record services?
   o No (1)
   o Yes (2)

Q6 Do you set goals for your herd production levels?
   o Yes (1)
   o No (2)

Q7 Do you usually meet your production level goals?
   o No (1)
Q8 Which of the following best describes your milking system?

- Stanchion system with a milk line (1)
- Elevated Parlor with a milk line (2)
- Rotary Parlor with a milk line (3)
- Robotic Unit(s)(4)
- Other (5) ___________________________________________________

**If you are using robotic milkers, please skip to question 13

Q9 Do you utilize automatic takeoffs on your machines?

- Yes (1)
- No (2)

Q10 Do your milkers palpate the cows’ udder to see if they are milked out before taking off the milking unit?

- Yes (1)
- No (2)
- They are supposed to, but I don’t think they do (3)

Q11 At your average milking session, how many cows are being milked simultaneously (for example, 6 cow parlor)?

______________________________________________________________

Q12 At your average milking session, how many milkers are working simultaneously (for example, 2 milkers in a 6-cow parlor)?

______________________________________________________________

Q13 Do you pre-dip your cows’ teats before milking?

- Yes (1)
- No (2)

Q14 If you pre-dip, what pre-dip solution do you use?

- Iodine solution (1)
- Chlorhexidine solution (2)
- Sodium Hypochlorite solution (3)
Q15 What kind of dipping cup/dipping system do you use?
   - Spray Bottle (1)
   - Ambic non-return teat dipper (2)
   - Moistened towelettes (wipe out) (3)
   - Regular mug/cup (4)
   - Other (3) ________________________________________________

Q16 What is the average time lapse between pre-dipping and placing the milking unit on the cow?
   - Under one minute (1)
   - 1-2 minutes (2)
   - 2-5 minutes (3)
   - The milking unit is placed based on when milk let down occurs (4)

Q17 Do you post-dip your cows after milking?
   - No (1)
   - Yes (2)

Q18 If you post dip, what post-dip solution do you use?
   - Iodine solution (1)
   - Chlorhexidine solution (2)
   - Sodium hypochlorite solution (3)
   - Dodecyl benzene sulfonic acid solution (4)
   - Other (5) ________________________________________________

Q19 What kind of dipping cup/dipping system do you use?
   - Spray Bottle (1)
   - Ambic non-return teat dipper (2)
   - Moistened towelettes (wipe out) (3)
   - Regular mug/cup (4)
   - Other (5) ________________________________________________

Q20 What is the average time lapse between taking off the milking unit and post dipping?
Q21 On average how much time lapses between the removal of the milking unit and the time the cow can lie down?
- Less than 5 minutes (1)
- 5-15 minutes (2)
- Over 15 minutes (3)

Q22 How often do your milkers change their gloves?
- They don't change their gloves (1)
- They change their gloves after every milking (2)
- They change their gloves after every cow (3)
- They don’t wear gloves (4)

Q23 Do they sanitize their gloved hands in between cows?
- Yes (1)
- Maybe (2)
- No (3)

Q24 If they sanitize their hands and/or gloves, what do they use? Please give product name and solution percentage

Q25 Do they change their gloves when moving from non-infected cows to mastitis cows?
- Yes (1)
- Maybe (2)
- No (3)

Q26 Do they milk the mastitis cows after all non-infected cows are milked?
- Yes (1)
- Maybe (2)
- No (3)

Q27 Are the mastitis cows housed separately from the non-infected cows?
Q28 Do you allow your milkers to milk if they are feeling ill?
- No (1)
- Yes (2)

Q29 What type of bedding do you use for your cows?
- Hay (1)
- Saw Dust Shavings (2)
- Sand (3)
- Other (4) ________________________________________________

Q30 What type of flooring do you have in your barn?
- Brick (1)
- Concrete (2)
- Other (3) ________________________________________________

Q31 Do your milkers complete other barn-related tasks while milking?
- Yes (1)
- No (2)
- I don't know (3)

Q32 Do you quarantine new pregnant heifers until after they have given birth before introducing them to your herd?
- No (1)
- Yes (2)

Q33 Do you send your cows to community/shared pastures?
- No (1)
- Yes (2)

Q34 How often does a bovine Veterinarian visit your farm?
Q35 Do you vaccinate your cows and heifers against *Staph aureus* mastitis (initial vaccine followed by boosters?)
- Yes (1)
- No (2)

Q36 Have you ever had a case of SA mastitis in your herd before?
- No (1)
- Maybe (2)
- Yes (3)

Q37 Were you able to get rid of the case of SA mastitis? (If you answered yes to Q36)
- Yes (1)
- No (2)
- Other______________________________
AUTHOR’S BIOGRAPHY

Morgan Belvin was born in Secaucus, New Jersey on February 11, 2000. She was raised in Rochelle Park, New Jersey, and graduated from Paramus Catholic High School in 2018. She attended the University of Maine as an undergraduate, majoring in Animal Veterinary Sciences and declaring a pre-veterinary concentration in her freshman year. Morgan has been a member of a variety of clubs and has been the President of the Maine Animal Club based at J.F Witter Farm. Morgan plans to attend veterinary school in Fall 2022 (as part of the Class of 2026), afterwards returning to Maine to practice as a large- and small-animal veterinarian.