



COLLEGE OF AGRICULTURE AND LIFE SCIENCES
DEPARTMENT OF ANIMAL SCIENCE

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With the recent completion of laboratory analysis, data entry and organization from samples gathered last winter and spring, we are now prepared to share the results obtained during our intensive bedding and mastitis risk study on 10 organic dairy farms. Five participating farms used a bedded pack system for housing their cows during the winter, and five farms used a tiestall barn with wood shavings or sawdust bedding. Although our study was interrupted due to COVID-19 before we were able to complete our goal of four farm visits for all 10 farms, we were still able to complete at least three visits for each farm. After taking stock of the data gathered during the summer, we came to the decision that we had sufficient information to answer the questions our study set out to explore. We would like to thank you for generously allowing us to visit your farm to conduct our research (especially during milking time!); with your participation, we will be closer to understanding the complex relationship between bacteria living in bedding material and a cow's risk of getting mastitis.

Included in this packet, you will find bedding culture results, bulk tank milk culture results, and quarter-milk culture results from all sampled cows from farm visits last winter and spring. We realize these individual results are roughly a year old. The amount of data generated from this study was enormous; in addition to bulk tank and bedding cultures from all visits, 8,400 quarter-milk samples were cultured, interpreted, entered into digital records, edited, and formatted into reports suitable for sharing. From these 8,400 cultures, over 1,300 bacterial isolates were obtained, all of which needed to be identified with lab tests and put into long-term storage. Thanks for your patience in sharing our results!

Although we couldn't share information in a time-frame suitable for making decisions on individual cows or truckloads of shavings, we hope this information can serve as a representative picture of udder health on your farm: although certain cows may freshen in and others get sold, the overall trends of where a farm is excelling with milk quality and what are some areas of opportunity can often remain the same. Additionally, although the bedding culture results are dated, having data from three different time points over the winter gives a good general idea of what kinds of bacteria are present in the bedding you use on your farm, and what range of numbers you can typically expect to see.

If you would like more time-sensitive diagnostic testing for your herd, we are happy to discuss further options with you. We encourage you to share these results with your herd veterinarian, and with your permission we would be happy to provide them with a copy. Again, thank you for participating. Our goal is to support organic milk production through animal health and milk quality research. We hope that you find this summary useful, and we encourage you to contact us directly with any questions. Caitlin can be reached by email at caitlin.jeffrey@uvm.edu, and John can be reached by phone at 802-656-1395, or by email at john.barlow@uvm.edu.

Sincerely,

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Organic Herds Bulk Tank Milk Analysis and Bedding Cultures

Introduction

Our lab is interested in quantifying the relationship between bulk tank milk quality, mastitis risk, and bedding material on organic dairy farms throughout the state. This 10-herd study represented the second phase of our three-year USDA project, where we visited five organic dairies in Vermont using a bedded pack to house cows over the winter, and five farms using a tiestall barn with wood shavings or sawdust. Farms were visited at least three times (except for one seasonal herd), with 4-6 weeks in between each farm visit. At each visit, quarter-milk samples were collected from 35 enrolled study cows in duplicate, as well as used bedding material and bulk tank milk. Teat skin swabs were also collected from enrolled study cows. A subset of these teat skin swabs will be submitted at a later date for analysis of the genetic material present to identify what kind of microbes inhabit the teat skin of cows. These teat skin swabs will not be analyzed using traditional aerobic culture techniques, as was done for bedding, bulk tank milk, and quarter-milk samples.

The bedding and bulk tank milk samples collected at your farm were sent to the Veterinary Diagnostic Laboratory at the University of Minnesota. The individual quarter-milk samples from cows on your farm were cultured in our lab at UVM.

How to use milk quality testing and the results from this analysis

Consistency is the key to milk quality. Serial testing is the best way to evaluate consistent milk quality. A single milk quality test panel should be interpreted with caution as one random test result may not reflect the overall trend. We recommend producers develop a routine bulk tank milk testing program to monitor trends in milk quality.

The bulk tank milk reports represent a snapshot from 3 (or 2, or 4) farm visits and may not accurately reflect your individual milk quality trends. The purpose of this study is to understand mastitis risk on a population of dairy farms. Care must be taken when evaluating the individual farm results from this study. We are providing your individual results so that you might compare them to the population averages and extremes. We are also providing a summary of the specific tests and how they can be used to diagnose milk quality problems.



Bulk Tank Analysis from Univ. of Minnesota – Methods and Interpretation

Adapted from “Interpretation and Use of Laboratory Culture Results” from Minnesota DHIA (<https://www.mndhia.org/uploads/5/0/3/6/50366013/interpretingcultureresults.pdf>) and University of Minnesota College of Veterinary Medicine Veterinary Diagnostic Lab Factsheets (<https://www.vdl.umn.edu/services-fees/udder-health-mastitis/factsheets-resources>)

Lab Pasteurization Count (LPC) colonies/mL: This is a count of the number of colonies of bacteria that are able to survive laboratory pasteurization at 143°F for 30 minutes. This is also called a pasteurized count. This value is expressed as the number of bacterial colonies formed per mL of milk (in other words, the number of colony-forming units or CFU per ml). *An LPC of under 100 is considered excellent; a count from 100-200 is acceptable, and a count of over 200 is concerning.* Because most mastitis-causing bacteria are killed in the pasteurization process, this test is a good indicator of function and cleanliness of the milking equipment and udder hygiene.

Standard Plate Count (SPC) colonies/mL: This is an estimate of total number of bacteria present in the bulk tank milk and able to grow in the presence of oxygen. *A CFU count of under 5,000 is considered excellent; a count of 5,000 to 10,000 is acceptable, and a count of over 10,000 is concerning.* Raw, total bacteria count, and aerobic counts are all different names and methods for counting total bacteria. Increases in the total bacteria count can be caused by poor milking hygiene, unclean or unsanitized milking equipment, improper cooling of milk, and cows with cases of mastitis shedding bacteria in milk.

Preliminary Incubation Count (PIC) colonies/mL: This is a measure of the bacterial colonies counted after the sample is allowed to sit at 55°F for 18 hours. This test measures bacteria in the milk that originate from the environment outside of the udder such as from soiled surfaces. These bacteria can often survive in cold environments. This count is generally higher than the RAW CFU count; however, if the PI count is 3 to 4 times higher than the RAW count, this is a concern. *Under 10,000 CFU/mL is considered an excellent count; 10,000-50,000 CFU/mL (or 3-4x the RAW CFU count) is acceptable, and over 50,000 CFU or over 3-4x the RAW CFU count is concerning.* PI counts above the concern level suggest more attention to cleaning of milking equipment surfaces or udder preparation or milk cooling is indicated. If a herd had excellent or acceptable LPC counts, herds with high PI counts might look into milk cooling deficiencies or challenges.

Coliform count (32 degrees C) colonies/mL: This count measures the colonies of coliform bacteria grown from the milk sample, incubated at 32 degrees C. Coliform bacteria can be found in several places, but are always found in manure: therefore, coliform counts can be a good indicator of the hygiene practices of the farm. Manure-contaminated bedding is a notorious source of coliform bacteria, and dirty teats can be a source of high coliform counts. High total coliform counts can also be associated with unclean milking equipment and milk residues on milking equipment. Coliform bacteria include *E. coli*, *Klebsiella*, *Enterobacter* and *Citrobacter*. *Under 10 CFU/mL is considered an excellent count; 10-50 CFU/mL is an acceptable count, and over 50 CFU/mL is concerning.* Identifying the species of coliform bacteria may help pin-point the cause of high counts.

Coliforms (colonies/mL): Coliform count is the number of colonies of *E. coli*, *Klebsiella*, *Citrobacter*, *Enterobacter*, and other related gram-negative mastitis organisms grown from the bulk tank milk at 37 degrees C for 48 hours (mimicking the temperature of the udder). This number can be thought of as a measure of the amount of potential coliform mastitis organisms, vs. “Coliform count (32 degrees C),” which more aims to estimate the number of bacteria that originate from manure or a contaminated environment. Coliforms are gram-negative bacteria commonly found in bedding, manure, water, and soil. A high coliform count can be caused by exposure to wet, dirty lots or contaminated bedding, milking wet cows or poor udder prep. *Klebsiella* in particular may be associated with the use of green sawdust or recycled manure bedding. Rates of new coliform infections are higher in the summer than in other seasons. Infection occurs when the teat end contacts contaminated material between milkings, and can cause life-threatening illness. Coliform counts can be reduced by improving stall (bedding) and lot sanitation, keeping cows standing after milking to allow for the teat sphincter to close, and keeping milking equipment in good order (avoiding air leaks and liner slips). *Less than 100 colonies/mL is considered an excellent coliform count, 100-400 colonies/mL is moderate, 400-700 colonies/mL is high, and above 700 colonies/mL is very high.*

Non-ag Strep colonies/mL: This is an indication of the colonies of *Streptococcus* and *Streptococcus*-like organisms (SSLO; *Streptococcus*, *Aerococcus*, *Enterococcus*, *Lactococcus*) grown from the bulk tank milk. The Non-ag streps include *Streptococcus uberis* and *Streptococcus dysgalactiae* as well as many other species of streptococci and enterococci. These organisms come primarily from the environment. Major sources of these bacteria are bedding material, manure, mud and infected cows. Infections with “environmental streps” frequently occur during the dry period, especially the first 2 weeks following dry off and the 2-3 weeks prior to calving. Minimizing exposure to the bacteria is essential for the prevention of environmental mastitis caused by non-ag Streps. Steps to achieve this include adequate amounts of clean, dry bedding in all stalls, and improved lot sanitation. Well-managed inorganic bedding (sand) is associated with fewer pathogens than organic bedding (straw, shavings, sawdust, etc.). Wet or soiled bedding should be removed at each milking. The back one-third of every stall should be bedded daily when using organic bedding material. Additionally, keeping cows standing after milking to allow for the teat sphincter to close, and keeping milking equipment in good order (avoiding air leaks and liner slips) should also help prevent infections with these environmental pathogens. *Less than 500 colonies/mL is considered low, 500-1200 colonies/mL is moderate, 1200-2000 colonies/mL is high, and above 2000 colonies/mL is very high.*

Staph. aureus colonies/mL: This is an indication of the colonies of *Staph. aureus* grown from the bulk tank milk. *Staph. aureus* is most commonly associated with cases of subclinical mastitis. Presence of this bacterium in the bulk tank milk suggests that at least one animal in the herd has a *Staph. aureus* intramammary infection. This organism is able to live in the udder without showing any physical (clinical) signs of mastitis. Cows with chronic *Staph. aureus* infections may show occasional clinical flare-ups of mastitis (abnormal milk). *Staph. aureus* is contagious (can spread from cow to cow, frequently on milking units, milkers’ hands, or contaminated common wash rags and towels), so efforts should be made to limit contact between cows. Good milking hygiene, including use of pre- and post-milking teat disinfection and separate towels for individual cows, is effective at reducing spread of *Staph. aureus*. Individual cow SCC can be used

to identify cows with subclinical mastitis, and individual cultures of high SCC cows can be used to identify the cause of subclinical mastitis. Repeated cultures are sometimes needed to determine the pathogen causing cases of subclinical or clinical mastitis. Cow composite or individual quarter culture or PCR test results can be used to support decisions on treating or culling cows. Culture can be an important component of *Staph. aureus* mastitis control. For example, in tie-stall barns *Staph. aureus* cows can be milked last (i.e., 'segregated' from other cows during milking). *Less than 50 CFU/mL is considered a low count, 50-150 CFU/mL is moderate, 150-250 CFU/mL is high, and over 250 CFU/mL is very high.* We will often observe a positive correlation between *Staph. aureus* counts and BTM SCC.

Most (7/10) of the farms participating in this study had at least one positive test for *Staph. aureus* in their bulk tank milk. If your herd was positive for *Staph. aureus* on any of these single tests, we believe this probably indicates that one or more of your cows has a *Staph. aureus* infection. *Staph. aureus* infections can remain undetected in many cows. Bulk tank milk testing cannot be used to predict how many cows are positive for *Staph. aureus*, however, if your *Staph. aureus* numbers are high, this could indicate that you may have several cows in your herd with *Staph. aureus* mastitis.

Staph sp. colonies/mL: This is an indication of the colonies of non-*Staph. aureus* Staph species grown from the bulk tank milk. There are over 50 different species of staphylococci that make up the group known as the Coagulase-negative staphs (CNS, or Staph species). Some of the most common Staph species isolated from mammary glands are *Staph. chromogenes*, *Staph. simulans*, *Staph. hemolyticus*, *Staph. epidermis*, and *Staph. sciuri*. Some Staph species are more pathogenic (disease-causing) than others, and vary in their effect on somatic cell count and milk production during an infection. Most often, clinical or subclinical infections caused by Staph. species will be relatively mild; quarters with subclinical infections usually don't exceed 500,000 cells/mL, and many of the cases will eventually self-cure. Persistent infections will likely clear during the dry period. Unlike *Staph. aureus* (spread from cow to cow), the CNS as a group are considered to be environmental pathogens. Staph species are commonly found on bovine skin and nasal passages, milkers' hands, and in the environment in bedding and manure. Staph species are primarily thought to be opportunistic pathogens, establishing infections when teat disinfection is not effective at milking. Controlling a high incidence of Staph species infections can be achieved by good pre- and post-milking teat disinfection with teat dip, and minimizing exposure to dirty environmental conditions (adequate amounts of clean, dry bedding in all stalls). *Less than 300 CFU/mL is considered a low count, 300-500 CFU/mL is moderate, 500-750 CFU/mL is high, and over 750 CFU/mL is very high.*

Strep. ag colonies/mL: This is an indication of the colonies of *Streptococcus agalactiae* grown from the bulk tank milk. *Strep. ag* is a contagious (spreads from cow to cow) mastitis pathogen that may be common on some dairies, but was not seen in any of the herds included in this study. Oftentimes only 10-15% of the cows infected with *Strep. ag* will exhibit signs of clinical mastitis, but most will have decreased milk production and high somatic cell counts. Purchased animals are frequently the source of bringing *Strep. ag* into the clean herd.

Mycoplasma culture: This was a culture to see if any *Mycoplasma* organisms were present in the bulk tank milk. *Mycoplasma* species are contagious organisms that may be found in infected udders, respiratory tracts, and urogenital tracts of apparently healthy animals. This disease is frequently brought onto a farm through the purchase of infected milking cows or heifers. Many *Mycoplasma* infections are subclinical and the infected cow has a low SCC. Mastitis caused by *Mycoplasma* does not respond to therapy. None of the herds included in the study had a positive *Mycoplasma* culture on bulk tank milk.

Prototheca culture: *Prototheca* is an algae commonly found in manure, soil and water, and wet areas in the cow's environment may pose a risk for infection. *Prototheca* infections occur when the teat end is exposed to very large numbers of this organism. Additionally, poor hygiene and contaminated intramammary infusion equipment (teat cannulas) can also be risk factors. *Prototheca* infections are most frequently subclinical, but infections can remain chronic, with a progressive decrease in milk production over time as the organism damages more of the gland. The cow-level somatic cell count may be only slightly elevated or may be greater than 1,000,000 cells/ml. None of the herds included in the study had a positive *Prototheca* culture on bulk tank milk.



Bedding Culture Analysis from University of Minnesota

Bedding cultures may be useful to evaluate “clean” bedding, to compare “before and after” (used/unused) bedding samples, or to evaluate bedding management. **These results are aerobic cultures done on used bedding material from your farm, with one sample taken at each farm visit.** Some farms used a different bedding material in the stalls where cows were milked, in addition to the material used for bedding- this is why you may see culture results for two bedding material types for each visit.

Recent work from the University of Minnesota looked at the relationship between bacteria counts in bedding, and a few different udder health measures (herd average linear score, proportion of the herd with mastitis/linear score above 4). From this work, they compiled various bacteria count cut-points, where udder health measures differed significantly above and below these cut-points. For some organisms (Staph species and Klebsiella), any detectable number of these bacteria was considered “high.” For coliforms and Streps and Strep-like organisms (SSLO, aka “Environmental Streps”), cut-points for relationships with udder health were found for low, moderate, and high counts. These cut-points are listed in the table below, which is from the University of Minnesota Laboratory for Udder Health Diagnostic Lab website.

Table 1: Sand (New or Recycled) or Organic Non-Manure Bedding (shavings, straw, etc.)

Unused	Low	Moderate	High
Coliform	≤ 500	-	>500
Klebsiella	0	-	>0
Staphs	0	-	>0
SSLO*	0	1-1,000	>1,000
Used	Low	Moderate	High
Coliform	≤ 10,000	-	>10,000
Klebsiella	0	-	>0
Staphs	0	-	>0
SSLO*	≤ 500,000	500,001-2,000,000	> 2,000,000

Table from University of Minnesota Laboratory for Udder Health, Bedding Culture Submission and Interpretation (https://www.vdl.umn.edu/sites/vdl.umn.edu/files/luh_bedding_factsheet.pdf)

*SSLO = Strep and Strep-like Organisms, aka “Environmental Streps”

Even though we now have evidence-based cut-points for bacteria counts in bedding, many other factors on an individual dairy could affect the relationship between bacteria in the bedding and udder health. Physical properties of the bedding itself or the environment (pH, moisture content, type of organic matter, ambient temperature and humidity) management factors (teat condition, efficacy of pre-milking teat disinfection), and the overall health and immune function of the herd in general can greatly influence the interaction between bacteria in the bedding and mastitis risk on a particular farm. Despite these other variables, these repeated bedding cultures from your farm can give you a good general idea of the range of bacteria counts and the types of bacteria normally present in used bedding on your farm during the winter months. If you have specific questions or concerns about what these bedding cultures may mean for your individual farm, these results could serve as a great starting point for discussion with your herd veterinarian or other advisor.

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Individual Quarter-Milk Results and Analysis - Methods and Interpretation

Included in this packet is the results for individual quarter-level milk cultures for all of the cows on your farm involved in the study. Quarter-milk cultures were done in our lab at UVM, with the intramammary infection status of each quarter determined using standard microbiologic methods. Quarter-milk cultures were done in duplicate, and bacteria isolated from infected quarters were identified using standard laboratory tests. All bacterial isolates from the quarter-milk cultures are also being identified down to the species-level by MALDI-TOF (matrix assisted laser desorption ionization-time of flight mass spectrometry); however, we likely won't have this information until Summer 2021. Our standard lab tests done are able to identify some bacteria down to species (i.e., *Staph. aureus* or *E. coli*), but only to species group for the Staph species (all non-aureus Staphs/CNS), Streps and strep-like organisms, and *Corynebacterium* species. We plan on sharing these more detailed, species-level results for bacterial isolates from your farm when they are finished (hopefully by Summer 2021).

The full quarter-milk results follow each cow over time, with the culture result of each of a cow's four quarters to the right of the cow ID and quarter. Quarter-milk was also submitted for somatic cell count; the right-most column lists the somatic cell count for that individual quarter-milk sample. Quarters will have listed next to them any species identified to be causing an intramammary infection; quarters with nothing listed next to them were deemed to be uninfected (no intramammary infection present). Some quarter-milk samples are labeled as contaminated; from these samples, we could not determine the infection status of a quarter.

We realize that this long list of individual culture results may be overwhelming! Additionally, given the age of these results, they are likely not representative of the current status of your herd (many of these infections may have cleared, cows have left the herd, heifers have freshened in, etc.). For this reason, **we have put together a one-page summary sheet with the total number of intramammary infections observed for each species group over the duration of the study, and identified some overall trends for udder health on your farm and possible opportunities for improvement.** The comprehensive list of quarter-milk culture results is included only completeness sake, if you are curious to see the full results.

Although many of the infections listed in these results may have cleared, there is one exception: quarters that were identified as having *Staph. aureus*. Infections with *Staph. aureus* can be chronic, sometimes living in the udder without showing any physical (clinical) signs of mastitis. As this mastitis pathogen is long-lived in the udder, contagious (passed from cow-to-cow), and can considerably increase somatic cell counts, **we've included a list below of cows with quarters we identified as having *Staph. aureus*.**

We realize that many of these quarters had previously been identified on the farm, and these cows were already segregated, milked last, or otherwise treated specially. Furthermore, in the time since these cultures were taken, some of these cows may have already left the herd if they were identified as having high cell counts or as *Staph. aureus* positive. **We hope that this list may still be useful if any of these cows are still on farm, so that you can keep a "close eye" on these quarters for elevated cell counts or an abnormal CMT, and work with your herd veterinarian or milk testing company to check if these cows are still positive for *Staph. aureus*.** Due to the

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nature of the organism, repeated cultures are sometimes needed to determine if *Staph. aureus* is present in a quarter, as it is often shed intermittently in the milk.

von Trapp Farm, *Staph. aureus* positive quarters by date

- ✓ **Leopard**
 - LH
 - 1.2.2020
 - 2.12.2020
 - 3.10.2020

Organism interpretation for quarter-milk cultures:

***Staph. aureus*:** *Staph. aureus* is most commonly associated with cases of subclinical mastitis. This organism is able to live in the udder without showing any physical (clinical) signs of mastitis. Cows with chronic *Staph. aureus* infections may show occasional clinical flare-ups of mastitis (abnormal milk). *Staph. aureus* is contagious (can spread from cow to cow, frequently on milking units or by milkers' hands), so efforts should be made to limit contact between cows. Good milking hygiene including use of post-milking teat disinfection is effective at reducing spread of *Staph. aureus*. Culture can be an important component of *Staph. aureus* mastitis control. For example, in tie-stall barns *Staph. aureus* cows can be milked last (i.e., 'segregated' from other cows during milking).

***Strep. species*:** This group of "non-ag Streps" includes all other Streptococcus and Streptococcus-like organisms (SSLO; *Streptococcus*, *Aerococcus*, *Enterococcus*, *Lactococcus*), including *Streptococcus uberus* and *Streptococcus dysgalactiae*. These organisms come primarily from the environment. Major sources of these bacteria are bedding material, manure, mud, and infected cows. Infections with these "environmental Streps" frequently occur during the dry period, especially during the first 2 weeks following dry off and during the 2-3 weeks prior to calving. These bacteria may cause subclinical mastitis with no apparent signs, or clinical mastitis with abnormal milk, swelling of the udder, and fever. Individual cow somatic cell counts are frequently elevated. Most of the infections caused by these SSLO's are eliminated by the cow's immune system or by antibiotic therapy if a clinical case of mastitis occurs. However, some environmental Strep. infections (18%) will become chronic and refractory or poorly responsive to treatment. Minimizing exposure to the bacteria is essential for the prevention of environmental mastitis caused by Strep. species. Steps to achieve this include adequate amounts of clean, dry bedding in all stalls. Well-managed inorganic bedding (sand) is associated with fewer pathogens than organic bedding (straw, shavings, sawdust, etc.).

***Staph species*:** There are over 50 different species of staphylococci that make up the group known as the coagulase-negative staphs (CNS, or Staph species). Some of the most common Staph species isolated from mammary glands are *Staph. chromogenes*, *Staph. simulans*, *Staph. hemolyticus*, *Staph. epidermis*, and *Staph. sciuri*. Some Staph species are more pathogenic (disease-causing) than others, and vary in their effect on somatic cell count and milk production during an infection. Most often, clinical or subclinical infections caused by Staph. species will be relatively mild; quarters with subclinical infections usually don't exceed 500,000 cells/mL, and

many of the cases will eventually self-cure. Unlike *Staph. aureus* (spread from cow to cow), the CNS as a group are considered to be environmental pathogens. Staph species are commonly found on bovine skin and nasal passages, milkers' hands, and in the environment in bedding and manure. Staph species are primarily thought to be opportunistic pathogens, establishing infections when teat disinfection is not effective at milking. Controlling a high incidence of Staph species infections can be achieved by good pre- and post-milking teat disinfection with teat dip, and minimizing exposure to dirty environmental conditions (adequate amounts of clean, dry bedding in all stalls). Staph species can be quite resistant to antibiotic therapy. However, most infections will resolve without treatment, given enough time. Persistent infections can often clear during the dry period. As there are many different species of CNS, and each can vary in its ability to cause disease and inflammation, each CNS positive culture result should be interpreted in light of the clinical symptoms or SCC history. All CNS isolates from this study are being identified down to the species level using MALDI-TOF (matrix assisted laser desorption ionization-time of flight mass spectrometry); however, we likely won't have this information until Summer 2021, but we will share these results with you when they're available.

Corynebacterium species: *Corynebacterium* species (Most frequently *C. bovis*) are contagious, Gram-positive pathogens that occasionally cause intramammary infections. This organism is commonly found in the environment and soil. *C. bovis* will usually cause only a mild increase in somatic cell count and a slight reduction in milk production. *C. bovis* will colonize the teat canal, so the teat canal as well as infected udders can act as reservoirs for infection. As this pathogen is contagious, it can spread from cow to cow at milking. Proper milking procedures, including the use of effective post-milking teat disinfectants, will help to reduce the number of new infections. After unit detachment, the application of a proven post-milking teat disinfectant should be applied with coverage over at least two-thirds of the teat barrel. The exception to this are post-dips with the active ingredient linear dodecylbenzene sulfonic acid, which are not effective against *C. bovis*. These infections often self-resolve during the dry period. New infections can occur at any time during lactation.

Gram-negative, coliform organisms: This group includes *E. coli*, *Enterobacter*, and *Serratia*. Gram-negative coliform bacteria are commonly found in bedding, manure, water, and soil. The most common route of infection for these organisms is fecal contamination of bedding and milking equipment, and rates of new infections are higher in the summer than in other seasons. Mammary gland infections with coliforms can cause life-threatening illness. Most of these infections occur during early lactation, or immediately after dry-off. Most *E. coli* infections are of short duration; approximately 50% last less than 10 days, but some may become chronic and last more than 100 days. Clinically, most severe coliform mastitis cases will look similar, with a sudden onset of fever, markedly decreased milk production, loss of appetite, and dehydration. Often these cows will go down and be unable to rise. Milk from the affected quarter may have large clots or be watery or bloody. In contrast, *Serratia* infections are typically less severe than those caused by *E. coli*, and most commonly chronic and subclinical. *Serratia* infections are most often mild and associated with an increase in a quarter's SCC, but cause no significant decrease in milk production. Immunization with coliform mastitis vaccines can reduce the incidence of coliform mastitis and severity of clinical cases if administered appropriately.

Klebsiella species: *Klebsiella* species are a specific type of Gram-negative, coliform organism. Like other coliforms, it can be found in high numbers in organic bedding material and manure. *Klebsiella* in particular may be associated with the use of green sawdust or recycled manure bedding. Animals infected with *Klebsiella* shed large amounts of the bacteria in their milk and manure, and transmission of the bacteria from an infected cow's milk on a milking unit to an uninfected cow has been shown to occur. Although clinical cases often resemble other kinds of coliform mastitis, *Klebsiella* infections are typically more damaging to the mammary gland than other coliforms and can become chronic, affecting the gland's milk production long-term. The infected animals or individual quarters usually have to be culled, as the cure rate is lower than that of other coliform organisms. Since *Klebsiella* infections may become chronic and subclinical, identifying and managing these cows is important to control transmission of the bacteria within the herd. As *Klebsiella* can act as a contagious mastitis pathogen, chronically infected cows should be segregated and milked last, and culled when possible. Just like other coliform organisms, maintaining a clean, dry environment is important to reduce exposure of the teat end to dirt and manure (frequent scraping of alleys and holding pens, keeping areas where cows lie down clean and well-bedded). Additionally, pre- and post-milking teat disinfection and good milking hygiene is important to minimize contagious spread at milking. Like other coliform infections, coliform mastitis vaccines are effective at reducing the severity of clinical cases.

Pseudomonas: *Pseudomonas* is a Gram-negative, non-coliform organism that only sporadically causes mastitis. It is very commonly found in the environment, and can be in contaminated water sources and teat dips, and can form biofilms on milking equipment. Exposure to small numbers of this bacteria causes chronic, subclinical infections, whereas exposure to large numbers can cause severe clinical cases. Chronically infected cows should be managed separately or culled, as *Pseudomonas* is difficult to treat.