SECTION II
MASTITIS – WHAT CAUSES IT AND HOW IT IS DETECTED

TABLE OF CONTENTS

1. MASTITIS AND ITS COSTS

1.1 WHAT IS MEANT BY THE TERM "MASTITIS"?

Fig. 1. Mastitic udder

Mastitis means inflammation of the mammary gland and usually results in a change to the anatomy and / or physiology of the udder. Inflammation is most often the animal's response to a microorganism that is causing disease (e.g. bacteria, viruses) but may also be a response to injury or systemic illness. Inflammation can be seen because the udder will become red, swollen and painful – indications that the immune system is fighting a microorganism. Inflammatory cells are usually white blood cells, and when present in the milk, are called somatic cells (which may also include a small number of mammary tissue cells). Inflammation in the udder is most often measured by the level of somatic cells (somatic cell counts or SCC) present in the milk.

"Intramammary infection" (IMI) is another term often used instead of the term mastitis but is limited to infectious causes only, i.e. those caused by a microorganism. For purposes of this course, we will use the term mastitis.

Mastitis causes a loss in milk production and the quality of the milk. It also affects the cheese-making quality. It can be detected by:

- Measuring the level of somatic cells present in the milk
- Clinical examination of the ewe, as well as
- Culturing the milk to identify the microorganisms responsible for the infection

Because mastitis is so important when trying to produce quality milk, this course will go into detail regarding the disease, how to detect it and how to treat and control it.

1.2 WHAT IS THE IMPORTANCE OF MASTITIS IN DAIRY SHEEP?

When you see an inflamed udder or abnormal milk in a ewe, you can recognize that this means lost milk and therefore income. There are also losses, both milk and income, associated with high SCC milk (subclinical mastitis).

1.2.1 ECONOMIC IMPACT

ESTIMATES OF THE ECONOMIC COST OF MASTITIS

To properly estimate the economic costs associated with mastitis, not only does a producer need to keep information on how much the disease is costing – but also determine what level of disease is acceptable and attainable (i.e. set a goal). Mastitis cannot be eradicated like some other diseases (e.g. maedi visna) and so the control program must be designed to reduce losses so that a good benefit – cost ratio can be achieved. Economists call this "**avoidable loss**", i.e. the difference between current losses and achievable losses given a good and affordable health management program.

Calculating the benefit – cost requires considering both the costs of having the disease versus not having the disease, i.e. changes to level of mastitis if specific interventions are taken, and the costs of lowering the incidence of mastitis. Below are examples of the costs (benefits) that go into this anlaysis.

COSTS ASSOCIATED WITH NOT CONTROLLING MASTITIS

Fig. 2. Cull ewes

- A ewe with mastitis is frequently culled earlier in life than a ewe that has not had mastitis. This changes the turnover rate of the flock with lost opportunity sales of replacement stock and having to replace a ewe in the most productive time of her life with a ewe lamb that will have lower milk production. Mastitis has been attributed as the reason for removal in up to 35% of cull ewes.
- If a ewe is culled early, then the difference between her value and that of a ewe raised or purchased to replace her.
- The difference between the value of a dead ewe (if any) and a replacement ewe, including the cost of disposal of the dead ewe.
- A ewe with mastitis will have lower milk production in terms of yield, and total solids (fat and protein) multiplied by the value of that lost milk.
- Penalties levied for high SCC, lost bonuses for producing high quality milk, or rejection of milk from processors if mastitis levels rise too high.
- Lost lamb production in terms of mortality differences and poorer lamb growth (for flocks that raise the lambs on the ewe).
- Veterinary costs for treating clinical mastitis, which includes drugs, equipment and labour.
- The value of discarded milk from treatments, plus the risk of inhibitors (drug residues) being detected in the milk or meat.
- Increased feed costs associated with feeding less productive ewes.
- Increased management costs (labour and facilities) associated with separating treated ewes or ewes with contagious forms of mastitis.

COSTS OF A FLOCK HEALTH PROGRAM DESIGNED TO CONTROL MASTITIS

- Improvements in housing (e.g. stocking density, ventilation).
- Improvements in milking equipment and its maintenance.
- Products to improve hygiene at milking (e.g. gloves, disinfectants, paper towels)
- Therapies such as “dry-cow” intramammary antibiotics that may cure existing infections and reduce new ones.
- Detection of and monitoring for subclinical mastitis (SCC and/or CMT and culturing milk from suspect cases).
- Costs associated with culling chronically infected ewes.

The difference between the costs of the disease + the cost of the interventions versus the financial improvements in the costs of disease from instituting the interventions is the benefit – cost ratio.

1.2.2 WELFARE

Mastitis is an illness that can cause permanent damage and sometimes death. It is also very painful to the ewe, particularly severe clinical mastitis. The welfare implications can also apply to offspring, as lambs nursing ewes with mastitis may starve to death. Mastitis must be properly treated and controlled to decrease the welfare costs to the farm.

1.2.3 PUBLIC HEALTH RISK

Many of the bacteria that can infect the udder of a ewe can also cause disease in humans that consume the raw milk product. Often these disease agents cause mastitis but sometimes they are shed in the milk and no signs of disease in the ewe or its milk are apparent.

Specific bacteria that are risky will be covered in Section II.3. While most of these microorganisms can be killed by proper pasteurization, raw milk cheeses or post-pasteurization contamination of milk products have been known to cause disease in humans.

Failure to withhold the milk after using antimicrobial drugs may leave residues of these drugs in the milk – increasing the risk of allergy or toxic insult in humans. Overtreatment with antimicrobials may increase the risk of antimicrobial resistance. Residues from other drugs, e.g. dewormers, lice treatments, hormones, painkillers, can all make humans ill.

2. SIGNS OF MASTITIS

Mastitis as a disease can be categorized into three types depending on the severity of the disease and the signs that it causes.

2.1 CLINICAL MASTITIS

Clinical mastitis presents itself with visible signs of infection with abnormal milk, and may or may not be associated with systemic signs, depending on the severity of the infection.

Fig. 3. Severe mastitis

2.1.1 SEVERE CLINICAL MASTITIS

This term means that the ewe has signs of illness, i.e. that the disease has affected it systemically. The incidence of severe clinical mastitis (number of cases/year) is generally ~ 5%, but the case fatality rate (proportion of ewes that die if they have the disease) is often 10 to 50%, making this not only economically important but also a welfare concern. Of those that survive, a very high percentage (as high as 70%) are culled either because the udder is irreversibly damaged or because of low milk production after recovery.

ACUTE SEVERE CLINICAL MASTITIS

Acute means sudden onset (as opposed to chronic where the disease has been present for weeks or months). Signs include: a high fever, usually 40.5 °C (105 °F) or higher (normal ~ 39.5 °C or 103 °F); depression; partial or complete lack of appetite; dehydration which is usually noted as sunken, dull eyes; and grinding teeth from pain. The udder or individual gland is swollen, hot, and painful to touch and usually has an inflamed or red appearance. The ewe may appear lame because of a reluctance to have the leg touch the udder. The milk may be watery in appearance or red-tinged from blood or appear like reddish serum - with or without clots of milk present.

GANGRENOUS MASTITIS

Fig. 4. Gangrenous mastitis

Gangrenous mastitis is often called "blue bag". Signs in the ewe may be similar to acute severe clinical mastitis with the exception that the gland and / or teats are cool or cold to the touch. About 8 to 10% of all cases of clinical mastitis are gangrenous. The onset of the disease is very acute, e.g. within a few hours. The skin is often bluish or purple in colour indicating that the blood supply to the skin and udder is damaged – usually from toxins released by the bacteria in the udder (Fig. 4). The secretion from the udder is usually scant, blood-tinged serum. Gas may also be "milked-out" and may sometimes be felt in the udder. The changes to the skin may advance beyond the udder and can affect the abdomen and inside of the thighs. When this is the case, the likelihood for survival of the ewe is poor.

Fig. 5. Sloughing gland

Gangrenous mastitis is most often seen within 1 to 4 weeks after lambing, and some cases occur after weaning – but it can occur at any time of lactation. The bacteria usually responsible for this type of mastitis are either *Staphylococcus aureus* (most commonly) or *Mannheimia haemolytica*, but other organisms are less commonly responsible, such as *Escherichia coli* (*E. coli*) or *Pseudomonas* bacteria (See Section II.3.2.1 for more details). Bacterial spores from clostridial organisms which prefer tissue with low levels of oxygen, may invade into damaged tissue causing "gangrene". Usually by the time the gland becomes cold to the touch, it is too late to save it – but perhaps not too late to save the ewe. However, ewes with gangrenous mastitis are usually very ill and in need of emergency treatment if they are to be saved.

Ewes that recover from gangrenous mastitis will lose the diseased gland (Fig. 5). The tissue dies and becomes purulent and the discharges from the decaying gland are full of bacteria. These wounds can easily become fly struck and filled with maggots. If not treated, the decaying material will be a source of infection to other ewes.

2.1.2 MODERATE CLINICAL MASTITIS

There are clinical changes to the udder and milk, but the ewe appears healthy and shows no illness.

ACUTE

Fig. 6. Firm gland

The onset is sudden, usually noticed from one milking to the next. The ewe is not ill but the udder is abnormal, e.g. the udder is uneven because one of the glands is swollen, the skin of the gland may appear pink or red and warm to the touch, the gland is often firm on palpation, or it may be lumpy or fibrotic (Fig. 6). With moderate clinical mastitis, the ewe will usually have a normal appetite, although milk production is reduced. The milk is likely abnormal in appearance, e.g. discoloured, clots or strings – sometimes purulent.

CHRONIC

Fig. 7. Ruptured abscess

Again the ewe is not ill but changes are noted in the udder. It may be that the initial infection was missed, or the ewe failed to recover completely from an acute case of mastitis. It is not uncommon for chronic mastitis to be noted when weaning the lambs, at dry-off, at shearing when the ewe is tipped up, or at lambing. The udder, one or both glands, is usually not of normal size – it may be larger because of the presence of scar tissue or abscesses, or shrunken because of the loss of functional tissue producing milk. It may be hard or lumpy. If abscesses are present, there may be pus draining from a hole in the gland (Fig. 7). The udder may still be warm to the touch and painful but these changes are mild compared to more severe mastitis. Usually the milk is abnormal and decreased in amount. It may even be absent or replaced with a purulent (i.e. pus-like) secretion.

2.1.3 MILD CLINICAL MASTITIS

Fig. 8. Normal appearing udder

The ewe is not ill, the udder appears normal but the milk appears abnormal. It may have clots, be purulent and / or have an abnormal colour or sometimes odour or taste. These changes may be acute or chronic but it may be more difficult to tell how long the changes have been present based on the appearance of the milk.

2.2 SUBCLINICAL MASTITIS

This is by far the most common presentation of mastitis. There are no clinical changes to the ewe or the udder and the milk has a normal appearance (Fig. 8). However, mastitis may be present and be causing an increase in somatic cells and a decrease in milk production. Subclinical mastitis must be detected using tests that detect either the somatic cells or by culturing the milk. Because most mastitis in ewes is subclinical it is economically critical to detect and control.

2.3 AGALACTIA

Agalactia means that one or both glands have no milk whatsoever. There are many causes of agalactia:

- The milk-producing tissue of the glands may be destroyed by an infection;
- The teats may have a blockage – either congenital (the teat wasn't formed correctly) or acquired from trauma (e.g. lamb bites), or secondary to severe mastitis;
- The ewe may not be producing milk either because she is ill, nutritionally starved, or she is at the end of her lactation.
- Apparent agalactia may occur if the milk let-down mechanism isn't working (e.g. stress or improper udder preparation), or the lambs have nursed out all of her milk.

3. WHAT MICROORGANISMS CAUSE MASTITIS IN SHEEP?

Most mastitis is caused by bacterial infection by a wide variety of bacteria. Viruses and yeasts may also be involved. Some of these bacteria are contagious, i.e. picked up from another animal – and some are contracted from the environment. The important and common pathogens are summarized in Table II.1. There are many other organisms which occur less commonly.

Table II.1. Classification of important udder pathogens of ewes

TYPE OF ORGANISM	NAME OF ORGANISM	CHARACTERISTICS
CONTAGIOUS (Sheep to Sheep)		
Bacteria	*Staphylococcus aureus*	Most common cause of clinical mastitis in ewes. Very difficult to cure. Highly contagious from other ewes, milkers' hands.
Bacteria	*Mannheimia* species (Pasteurella)	Common cause of clinical mastitis in ewes. May come from throat of nursing lambs.
Bacteria	Coagulase negative staphylococcus (CNS)	A group of organisms which are the most common cause of mild clinical mastitis and subclinical mastitis
Bacteria	*Streptococcus agalactiae*	Occurs more commonly in cattle. Rare in sheep.
Mycoplasma	*Mycoplasma agalactiae*	Very rare in North America but common cause of mastitis in European sheep and goats. Uncurable.
Virus	Maedi visna virus (Ovine lentivirus)	Common infection in Canadian sheep. Causes inflammation and scarring of the udder with lower milk production.
Prion	Scrapie	Is shed in the milk but cannot be detected.
ENVIRONMENTAL (Environment to Sheep)		
Bacteria	*Streptococcus dysgalactia* *Streptococcus uberis*	From dirty environment and udder. Can also be transmitted ewe to ewe (contagious) and causes joint infections in lambs and abortion in ewes
Bacteria	Coliforms (E. coli)	From dirty environment. Common in dairy cattle. Less common in sheep.
Bacteria	*Pseudomonas aeroginosa*	From dirty water. Also contagious ewe to ewe. Incurable.
Bacteria	*Listeria monocytogenes*	From wet condition, rotting feed. Can be shed in the milk without signs of mastitis. Important public health issue.
Yeast	*Candida albicans & Cryptococcus*	Usually from overtreatment with antibiotics.

3.1 CONTAGIOUS ORGANISMS

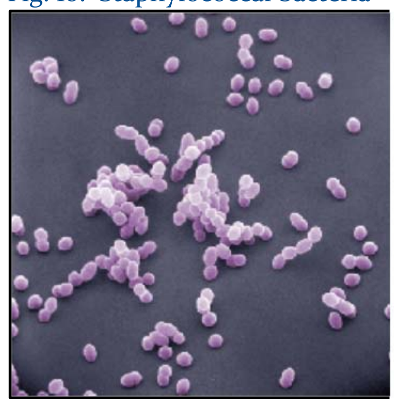
Contagious organisms are pathogens transferred from animal-to-animal. This transfer of bacteria is usually done at milking, from sources such as milker's hands (Fig. 9), towels that are used on multiple ewes, or from milk remaining in liners of the milking machine. These infections tend to be a problem on-farm, as clinical infections are easier to treat, but the pathogens will remain in the udder, leaving the ewe's SCC levels to be consistently high, as compared with unaffected animals.

3.1.1 BACTERIA

STAPHYLOCOCCUS AUREUS

Fig. 9. Hands may carry contagious bacteria

Fig. 10. Staphylococcal bacteria



Staph. aureus is a contagious pathogen, which is an important threat to udder health in many flocks. It is generally transferred to ewes at milking from both the milk and teat skin of infected animals, and is transferred to uninfected animals. This transfer is done through milking equipment, milker's hands and from towels used to dry the udder if they are used on multiple ewes. At the initial stages of infection, *Staph. aureus* can cause clinical mastitis; however, most commonly *Staph. aureus* is a subclinical infection, but often with elevated SCC counts. Although these infections are generally subclinical, they are chronic, cause loss of milk production and are very hard to treat. The infection often results in the formation of small abscesses in the udder tissue, making it difficult for antibiotics to reach the bacteria.

Staph. aureus is identified through culturing of milk from suspected cases. Antibiotic treatment during lactation has low success of curing the infection; however, treatment during the dry period holds promise. Most importantly, the most effective way to control this pathogen on-farm is to take stringent prevention protocols when milking. These management practices include milking *Staph. aureus* infected animals in the group last, and thoroughly disinfecting the milking equipment after use on these animals, so pathogens are not transmitted to uninfected ewes. In some cases, if *Staph. aureus* is a major issue in a flock, a separate milking unit can be reserved for milking these problem animals.

MANNHEIMIA SPECIES (PREVIOUSLY *PASTEURELLA*)

Mastitis caused by *Mannheimia* bacteria is relatively common among sheep flocks, and results in severe clinical cases of mastitis. The strains of this pathogen that are isolated from the teat skin of infected udders are identical to those isolated from the throat area of lambs and the same strains which cause pneumonia. This association suggests that infections are transmitted to ewes from nursing lambs, which can be very detrimental early in lactation. Damage to the teat seems to be necessary for these bacteria to cause mastitis.

COAGULASE-NEGATIVE STAPHYLOCOCCI (CNS)

Coagulase-negative staphylococci (CNS) are a group of pathogens that are generally characterized as one type of pathogen. This characterization is done because in general, these species of CNS (which spans to over 50 different species) affect ewes in generally the same manner. Coagulase-negative staphylococci commonly present themselves in a subclinical form, and can be quite persistent in the udder. Although these bacteria have historically been considered as contagious, some may actually be also contracted from the environment.

Coagulase-negative staphylococci are identified through culturing milk from suspected cases. Most laboratories do not determine the actual species of the bacteria routinely as this is expensive and tends not to change the approach to treatment. Research has shown that some of the more prevalent CNS species in flocks are *Staph. epidermidis* (often found in human skin infections) and *Staph. simulans*.

STREPTOCOCCUS AGALACTIAE

Although a **very rare** infection in sheep, *Streptococcus agalactiae* can be transferred to ewes during milking. If this infection is identified on-farm, it can be eradicated from the flock through treatment of affected ewes during lactation. Elevations in SCC levels are a good indication of *Strep. agalactiae* infections, and can be a key identifier of subclinical infections.

Strep. agalactiae are identified through culturing milk from suspected cases. Unlike *Staph. aureus*, with *Strep. agalactiae*, as antibiotic treatment can successfully kill these pathogens, and rid the infection from the udder.

3.1.2 MYCOPLASMA

MYCOPLASMA AGALACTIAE

This particular bacterium, which causes a disease called “contagious agalactia”, is considered not to occur in North America but is a common and important cause of mastitis of sheep and goats in Europe. *M. agalactiae* does not respond to treatment. Contagious agalactia syndrome can also cause arthritis and conjunctivitis; however, it is most importantly a cause of mastitis in ewes. Routine milk culturing will not grow these bacteria. If mastitis in the flock is severe and other bacteria are not isolated from milk samples, your flock veterinarian may decide to culture the milk for mycoplasma, which requires special milk culturing methods.

3.1.3 MAEDI VISNA VIRUS

Maedi visna is a very common disease in dairy sheep in Canada. It is caused by a slow virus (maedi visna virus – small ruminant lentivirus). Animals can be infected at any age but infection as lambs likely has the most effect on the ewe later in life. It becomes infected through the colostrum, from respiratory secretions and sometimes while the lamb is still in the uterus. The sheep remains infected for life and there is no cure.

Fig. 11. Ewe with maedi visna

The virus targets the udder and lungs. As mastitis, it causes uniform hardening of the udder and loss of milk. The virus causes an influx of lymphocytes into the udder tissues, which causes severe inflammation, scarring and loss of milk producing alveoli. Recent research suggests that infected ewes produce as much as 15% less milk than healthy ewes. Ontario has a voluntary program designed to help producers eradicate this important and common infection, and remain low risk[1]

[1] http://www.uoguelph.ca/~pmenzies/mv/OMVFSP_Index.html

3.1.4 SCRAPIE

Fig. 12. Ewe with scrapie

Scrapie transmitted through milk can be a concern in dairy flocks, but does not cause mastitis. Prions, which are the infectious proteins that carry scrapie in the body, have been isolated from the brain, tonsil and lymph nodes, as well as mastitic mammary glands, which suggest that these prions can be transferred to the ewe's milk. This could pose a problem with the transfer of these prions from a ewe's milk or colostrum to her lambs, which could increase the potential of scrapie in these young animals. At this point, scrapie is not considered to be a zoonotic disease (i.e. doesn't infect humans). Canada has a voluntary scrapie certification program[2]. To assure that this disease is not allowed into the flock, only sheep from certified flocks should be purchased.

3.2 ENVIRONMENTAL ORGANISMS

Environmental organisms are pathogens that are transmitted by the environment into the udder. These pathogens are typically found where the animals are housed, and can be transmitted through manure, bedding, or even water sources. This group of pathogens tend to cause acute, and sometimes severe, cases of mastitis, with an increase of SCC during the time of infection, but tend to cure quite effectively with the use of antibiotics.

3.2.1 BACTERIA

STREPTOCOCCUS DYSGALACTIA

Infection from this bacterium is not as common as those caused by CNS or *Staph aureus*. Although *Strep. dysgalactia* is classified as an environmental pathogen, it does have some contagious characteristics. This pathogen has been isolated from the teat skin, but tends to be quite present in the environment as well. It has also been shown to be transmitted by flies, and infections tend to flare up during the warmer summer months. Treatment with intramammary products containing penicillin has been shown to be an effective intramammary treatment for *Strep. dysgalactia*. Consult your flock veterinarian on the best way to approach management of this infection.

Strep. dysgalactia can cause other problems in sheep, notably it is an important cause of infectious polyarthritis in lambs. Infection occurs shortly after lambing, especially in poor environmental housing conditions. The bacteria enter the bodies of the lambs through a wide potential of entries, and subsequently enter the bloodstream, targeting the joints. Lambs with polyarthritis generally fair poorer in condition than healthy lambs, and are very lame.

STREPTOCOCCUS UBERIS

Strep. uberis is a common problem in both cow and goat milking operations and less common in sheep. Much like *Strep. dysgalactia*, *Strep. uberis* was once thought to be solely environmental, however, current research has shown that there are some contagious characteristics for different

[2] http://www.scrapiecanada.ca/certification.html

strains of this pathogen. These infections cause a very acute and sometimes severe case of clinical mastitis, with severe udder swelling and redness, and high body temperatures.

This pathogen is identified through milk culturing. It is important to treat these infections quickly with antibiotics; however, the cure rate is not always favourable. Infections have the possibility to become subclinical, and can subsequently be transmitted to other animals.

COLIFORM BACTERIA

Although unusual in sheep, coliform infections can potentially cause severe cases of mastitis, with both local signs of inflammation in the udder, as well as systemic signs throughout the body. Common coliform bacteria include *E. coli* and *Klebsiella pneumonia*. As these infections are extremely sudden and severe, antibiotic treatment of the intramammary infection may not be effective, however, treating the systemic signs of pain and dehydration are essential to lessening the adverse effects on the ewe. Coliform mastitis is a veterinary emergency. Salmonella bacteria are an uncommon infection in sheep and pose most risk because the bacteria can be transmitted through consumption of unpasteurized milk and cheeses.

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa bacteria are classified as environmental coliforms; however, they also have some contagious properties. This pathogen is found in contaminated water sources, which could be present anywhere on-farm, but can commonly be isolated from wash water in the milking units. These cases of mastitis lead to extreme cases of infection, and can present itself in acute, toxic cases, or chronic cases, with elevated SCC. Antibiotic treatment is rarely successful; therefore it is common to cull these animals from the flock, to both reduce SCC levels from chronic cases, and to decrease the potential for pathogens to be contagiously transferred to other ewes.

LISTERIA MONOCYTOGENES

Listeria monocytogenes is the cause of the zoonotic disease, listeriosis. Transmission of this disease is through feeding poor quality ensiled forages, usually with evidence of spoilage and contamination of raw milk, most frequently seen when the milk is unpasteurized (see Section I.2.4.6). The bacteria are often shed in the milk without signs of infection. However, in some cases, *Listeria* can be the cause of mastitis in ewes. It can also cause disease of the nervous system and abortion in sheep. *Listeria* is more often present as a subclinical infection, and causes slight local swelling in the udder, but infected ewes generally do not show any systemic signs. *Listeria* bacteria are shed continually throughout the time of infection, with elevated SCC. It is important to monitor and control these cases of *Listeria* mastitis, as it could potentially be transferred to humans through consumption of unpasteurized milk and cheeses, or by post-pasteurization contamination of milk.

3.2.2 YEAST

Mastitis caused by yeast is not common, however, if it does occur; it is very difficult to manage. Yeasts are environmental organisms, and are frequently found in water sources. They have an increased potential for causing an intramammary infection during milking if the teats are wet when the milker is put on. Overtreatment with antibiotics or poor hygiene when inserting mastitis ointment tubes is commonly associated with outbreaks of yeast mastitis. Yeast infections present as acute clinical infections, with high body temperatures in ewes. Yeast mastitis does not respond to standard antibiotic therapy and antibiotics should <u>not</u> be used to treat infections. However, the symptoms of these infections could be addressed with the use of pain management therapies and frequent stripping of the affected glands.

4. IMPORTANT RISK FACTORS FOR MASTITIS

There are many factors that predispose a ewe to developing mastitis or influence how common mastitis is in a flock other than the presence of pathogenic microorganisms. These are summarized in Table II.2 and are covered in more detail in the following sections.

Table II.2. Risk factors for mastitis in dairy ewes

FACTOR	RISK
Lambing Time	Weakened immune system; number of lambs born; difficult lambing.
Stage of Lactation	Ewes will have a higher incidence of infection when lambs are allowed to nurse; and the prevalence of infection increases in late lactation.
Nursing Lambs	Teat biting; poor emptying of the gland from uneven nursing.
Dry-Off	Stress of separating lambs; timing and method of dry-off.
Lactation Number	Older ewes tend to be more at risk of mastitis.
Viral Infections	Maedi visna; contagious ecthyma (orf, soremouth).
Udder Shape and Size	Poor shape interferes with milk-out; poor size will reduce milk production; poor teat placement will interfere with milk-out.
Teats	Teat end calluses from over-milking or long milk-out times; warts; contagious ecthyma; bites.
Environment	High stocking densities; poor ventilation; wet and cold floor and dirty bedding; air temperature too hot or cold; high humidity; inclement weather; relocating and mixing ewes.
Milking Technique and Equipment	Poor udder preparation – cleanliness and milk let-down; dirty hands; cracked and worn teat liners; high vacuum levels; inadequate vacuum reserve; incorrect pulsation rate and ratio; over-milking.
Genetics	Resistance to mastitis; heritability of SCC levels.
Nutrition	Low energy; selenium and vitamin E.

4.1 STAGE OF LACTATION

4.1.1 LAMBING TIME

Fig. 13. Nursing lambs

Research has shown that high producing dairy ewes have more poorly performing immune systems than meat-type ewes around lambing. This may translate into increased susceptibility to mastitis or other diseases.

4.1.2. STAGE OF LACTATION

Ewes that have first nursed lambs before entering the milking string have a higher prevalence of mastitis at the beginning of their exclusively machine-milked lactation – suggesting that nursing lambs are important in the risk of mastitis (See Section II.4.2). SCC levels increase when milk production starts to decrease – partly due to volume and partly because the udder is starting to involute through apoptosis (see Section I.1.2.3), when macrophage type cells congregate to "clean up" cellular debris.

4.2 RISK FROM NURSING LAMBS

The risk factors for mastitis from nursing lambs are multiple and are outlined below.

Fig. 15. Bite wounds on teats

4.2.1 TEAT BITING

Several studies have noted that nursing lambs will bite teats, sometimes breaking the skin or even penetrating the streak canal of the teat. While the reason for this behaviour is not completely understood, it is speculated that it could be because the ewe is producing insufficient milk for the lamb, or that multiple lambs are competing for teats.

Fig. 14. Uneven udder

4.2.2 POOR EMPTYING OF A GLAND

If a ewe is nursing a single lamb, that lamb may favour nursing one gland over another. Poor emptying of a gland can increase the risk of clinical mastitis in cattle, and it is likely also true for ewes. Also, because of the FIL protein (see Section I.1.2.3) – the gland will start to involute even if the other gland is continually emptied.

4.2.3 SEPARATING LAMBS DURING MILKING

When lambs are separated from ewes for milking, there is a measurable stress to the ewes, including changes in the immune system. The effect of this stress with respect to milk yield and mastitis needs to be assessed further.

4.3. DRY-OFF AT END OF LACTATION

Dry-off should be done when the ewe's milk production has dropped below a specific level, which differs between breed and farm. Ewes at this point are undergoing a natural involution of the udder and the stress involved with milk cessation is mild. Dry-off should be done <u>suddenly</u>, when milk production has dropped sufficiently. A keratin plug forms within a few days of dry-off in the streak canal of the teat, which protects against mastitis bacteria from entering. Removal of this plug because of milking out a "full" udder has been associated with dry-period mastitis.

4.4 LACTATION NUMBER / PARITY

First fresheners tend to have lower SCC values in the face of mastitis. Older ewes are more likely to have mastitis because of increased exposure to risk factors and changes to the anatomy of the udder and teats. Ewes 4 years and older are more likely to have elevated SCC counts (as measured by CMT) compared to younger ewes.

4.5 VIRAL INFECTIONS

4.5.1 MAEDI VISNA VIRUS

As mentioned in Section II. 3.1.3, MVV causes mastitis in ewes with the presence of other bacteria. What is not clear is if MVV will predispose the udder to bacterial mastitis. Regardless, MVV infection is an important source of lost revenue in milk production.

4.5.2 CONTAGIOUS ECTHYMA (ORF) INFECTIONS

Contagious ecthyma, also called "orf", "scabby mouth" and "sore mouth" is a common viral infection of sheep and goats. Lambs are most susceptible to disease but it can also be seen in adult sheep, particularly if not previously exposed. The virus prefers to infect regions of the lips and nose but can commonly infect the teats, coronary band above the hoofs, inside the mouth, around the eyes, tips of the ears and around the vulva or penis. Rams often get infected in the poll region, particularly if they fight. The lesions are raised and red, often covered with purulent debris and a scab and last about 6 weeks. Although the infection is painful, usually lambs handle it well.

Fig. 16. Contagious ecthyma infection

Unfortunately the lesions almost always become infected with *Staph. aureus* bacteria. This increases the risk of mastitis to the ewe in one of two ways: either the lamb nursing with infected lip or mouth lesions bathes the end of the teat in *Staph.* bacteria increasing the risk of mastitis, or the ewe develops orf on her teat and then is at greatly increased risk of *Staph. aureus* mastitis. Either is very dangerous to the ewe.

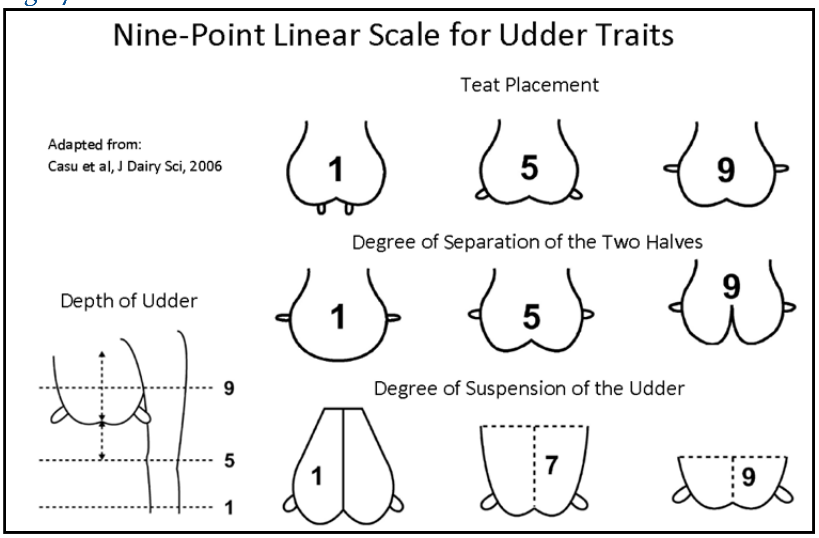
4.6 UDDER SHAPE AND SIZE

Quite a bit of research has been done on udder shape and size, and milk yield. Scoring systems have been developed for producers trying to select superior ewes to milk. Cistern size means that the ewe can store more milk between milkings allowing for less frequent milking after mid-lactation. Conformation of the udder means more efficient milk-out, allowing less residual milk and the need for machine-stripping. Caja et al, 2000 summarized the attributes of a good dairy sheep udder:

- Great volume, with globular shape and clearly defined teats
- Soft and elastic tissues, with palpable gland cisterns inside
- Moderate height, not surpassing the hock
- Marked suspensory ligament
- Teats of medium size (length and width), situated near to vertical

Fig. 17 is adapted from one of the 9-point systems developed for assessing udder conformation. Teat placement for machine milking should be closer to 1 in the scale of 1 to 9. However, keep in mind that what is optimal for the milking machine is not optimal for the lamb. Separation of the two glands should be closer to 9 on a scale of 1 to 9. Suspension and depth of the udder should be closer to 9 on a scale of 1 to 9. Degree of suspension and the depth of the udder appear to be highly correlated. Missing from this system represented below, which is usually present in other systems, is scoring of teat length (1 = short; 9 = long).

Fig. 17.



4.7 TEATS

Teat damage caused by nursing lambs, contagious ecthyma lesions and chapping have been discussed previously in Sections II.4.2.1 and II.4.5.2. The teat is an amazing structure – with the teat sphincter and lymphoid follicles just inside the teat cistern doing an amazing job keeping mastitis pathogens from invading further into the udder.

Other important teat conditions are listed below:

4.7,1 TEAT END CALLUSES

Quite a bit of research has been done in dairy cows with respect to mastitis and presence of teat end calluses – but none yet in dairy ewes. In cows, calluses are caused by:

- Pointed teats, as opposed to inverted or flat-ended
- Longer machine-milking times
- Low milk-flow (over-milking)
- Irritating chemicals used for udder washing
- High vacuum levels
- Factors associated with teat cup liners

Fig. 18. Teat end callus

These calluses can be seen around the teat orifice and vary from a smooth ring to a rough ring with severe proliferation of tissue (hyperkeratosis). In dairy cattle, presence of hyperkeratosis has been linked to an increase risk of *Staph. aureus* mastitis. More work needs to be done in dairy ewes and the relationship to milk-out time, but we can learn from work already done in cattle.

Fig. 19. Warts on teats

4.7.2 WARTS

Warts are caused by a papilloma virus and causes cauliflower like growths on the teats. These growths may interfere with proper application and function of the teat cups, and they may become infected with bacteria. If they occur close to the teat orifice, they may increase the risk of mastitis. They are also contagious to other sheep.

4.8 ENVIRONMENT

The environment that the dairy ewe is maintained in has a profound effect on milk production. Poor environment increases the risk of environmental causes of mastitis, and increases the stress to the ewe. The information is summarized in Section I.2.3 - Table I.4.

4.9 MILKING TECHNIQUE AND EQUIPMENT

4.9.1 UDDER PREPARATION

Regardless of how clean the environment is, there will be bacterial contamination of the udder and teats. Improper cleaning and drying will lead to opportunities for these bacteria to enter the teat during or after milking and cause mastitis.

In some jurisdictions dairy cows must have hair removed from the udder and teats to lower the risk of bacterial contamination. Sheep udders may have hair but some may also have wool, depending on the breed. Wool from the tail, escutcheon, thighs, and inside of the legs may also pose a risk. Feces, urine and birth fluids can contaminate this wool and skin. Ewes should be crutched at the beginning of each lactation to facilitate easy cleaning of the udder and remove the risk of manure tags contaminating the udder, hands and milking equipment (see Section I.2.1.5).

4.9.2 HANDS AND HAND-MILKING

Hands are easily contaminated with bacteria which can be transferred to the teats and milking equipment. These bacteria may be plentiful on the hands, even if they appear superficially clean. Wounds and cracks on the hands are often infected with mastitis causing bacteria, particularly *Staph. aureus* and CNS. Hand milking is, unfortunately a very efficient way to transfer mastitis pathogens from one ewe to the next. Because it is sometimes impossible to remove all risky bacteria from the hands – even with frequent washing with disinfectant soaps, it is advisable to wear disposable gloves. If gloves become soiled, change gloves or wash in a disinfectant soap and then dry.

4.9.3 MACHINE MILKING

Set-up and maintenance of milking equipment is covered in more detail in Section IV.3.

TEAT CUPS

Fig. 20. Liner cracks with bacteria

Teat cups are well recognized as a common source of contamination with mastitis pathogens. Tiny cracks in the liners can harbour a teeming population of bacteria, which will mix with milk and impact the teat sphincter with a soup of microorganisms (Fig. 20).

VACUUM LEVELS

Recommended vacuum level for dairy ewes at the teat level is between 32.5 to 39 kPa (9.6 to 11.5 inches of mercury (Hg)) although recommendations vary depending on the type of system used, e.g. 35.6 kPa (10.5 inches Hg) for lowline systems and 39 kPa (11.5 inches Hg) for highline. Overly high levels of vacuum can damage the teat end. Acutely this may cause problems with the return of blood flow back to the teat after milking, and for teat sphincter closure. Chronically, it may cause damage to the teat end – rings of scar tissue, which can harbour microorganisms that cause mastitis. However, too low vacuum levels may increase the risk of clusters dropping off and increase the risk of impacts (see below).

VACUUM RESERVE

Having sufficient vacuum reserve is critical to prevent changes in direction of milk flow. When milking units are removed incorrectly or fall-off and vacuum levels drop, the milk from another ewe may backflow and hit the teat end with a spray of milk – which is open and susceptible to infection. If the milk is contaminated with contagious mastitis pathogens, the ewe is at risk of infection. Squawks, which indicate sucking of air around the teat cup and into the milk-line, can have a similar effect. Crimped-over milk lines to accommodate milking of ewes with a blind gland or a gland with less milk (usually a ewe that has mastitis), can also suck air – causing hits or impacts of milk on the teat end.

PULSATION RATE AND RATIO

The recommended pulsation rate for dairy sheep is between 90 to 180 cycles / min with a ratio of between 50 to 60% (International Dairy Federation, 2002). The higher rate of 180 cycles/min appears to have no detrimental effect on teat thickness or udder health when milked at a vacuum pressure of 36 kPa.

MILKING TIME

Sheep milk out very quickly, often in less than 2 min. Over-milking will tire the teat sphincter and over time will damage it. If the teat sphincter doesn't close properly after milking, there is an increased risk of bacteria invading the udder and causing mastitis.

Reasons for over-milking include:

- Too many units per milker so that the milker cannot manage udder preparation and unit management in a timely manner
- Improper stimulation of the udder for optimal milk ejection (milk let-down);
- Issues in the parlour that interfere with milk ejection e.g. too noisy, yelling and pushing

4.10 GENETICS

Levels of somatic cells and presence of bacterial infections are two ways in which researchers determine mastitis in a ewe for purposes of evaluating their ability to resist this disease.

4.10.1 LEVELS OF SOMATIC CELLS

Elevated somatic cell count (SCC) levels have been used to select against ewes that are susceptible to mastitis, however SCC levels are lowly heritable – with estimates of between 0.04 and 0.24h^2. This means that selecting ewes for low SCC values would make for slow progress in “resistance” to mastitis. In comparison, heritability of milk yield is 0.34, milk fat – 0.50, and milk protein – 0.63.

There is mixed evidence that in dairy ewes SCC and milk production are negatively correlated (i.e. high producing ewes are more likely to get mastitis). Regardless, the effect – if present – is much milder than seen in dairy cows, where high producing cows appear to be much more susceptible to mastitis than low producing. This difference could be because the dairy ewe has not been genetically selected as intensively as dairy cows – yet.

4.10.2 RESISTANCE TO CLINICAL MASTITIS

There is on-going research both in dairy cows and sheep looking for genetic markers and other indications of resistance and susceptibility to episodes of clinical mastitis. Most are based on the animal’s ability to fight infection.

4.11 NUTRITION

4.11.1 LOW ENERGY

In early lactation, ewes are often in a negative energy balance, which must be compensated for by improved quality and quantity of feed during this period and by having an adequate body condition score at the beginning of the period (covered in more detail in Section I.2.4). Underfed ewes in late gestation and early lactation have been shown to have increased SCC and changes to the fatty acid profile of the milk.

Being pregnant with multiple lambs appears to increase risk – possibly because of increased nutritional needs but perhaps because of reduced function of the immune system and therefore the ewes are less able to ward off infection. Because ewes carrying multiple lambs produce more milk, and the extra lambs provide additional income – it is necessary to make sure the environmental hygiene is excellent for these animals. It is important to note that both overfeeding and underfeeding of pregnant ewes can have a negative effect on udder size, birth weights of the lambs, and total levels of antibodies available in the colostrum.

4.11.2 SELENIUM & VITAMIN E

In areas where selenium is deficient – which is most of Canada, it is critical to supplement the ewes’ diet during gestation as well as lactation. Vitamin E, while present in green pastures in large amounts, degrades quickly in stored feeds and so must be supplemented to ewes that are not on green pasture grazing. Both are important in prevention of mastitis and general udder health. This is covered in more detail in Section I.2.4.

5. HOW DO WE DETECT MASTITIS IN AN INDIVIDUAL SHEEP?

5.1 EXAMINATION OF THE UDDER AND TEATS

5.1.1 UDDER

When a gland has a clinical mastitis infection, the signs are usually obvious; the affected gland or half is often enlarged and swollen. If the infection is severe enough, the udder is red in colour (indicating that it is inflamed), and is hot to the touch. Ewes are susceptible to a condition called "blue bag" in which the toxins of the bacteria (usually *Staph. aureus* but sometimes *Mannheimia* or *Pseudomonas*) cause the tissues of the gland to die and become gangrenous. The gland is cold to the touch and blue and the ewe is very ill. If the infection is chronic, the udder can be shrunken, hard and may contain lumps or abscesses (Fig. 21).

Fig. 22. *Staph. spp.* impetigo

Maedi visna virus targets the udder, but symptoms are different from bacterial infections. The udder is often called "hard bag", and appears as though it is full with milk, however, it is quite hard, and very little milk can be removed from the gland.

Fig. 23. Teat bite wound

Staphylococcal impetigo is an udder skin condition that is associated with *Staph. aureus* or other *Staph.* bacteria, which are common causes of clinical mastitis in sheep. This develops a rash with small bumps on the udder, and if not treated, can possibly allow bacteria to enter the udder through the skin (Fig. 22).

5.1.2 TEATS

Teat end health is very important to help control udder health in sheep. The conditions of the teats are discussed in Section II.4.6 and II.4.7. Poor vacuum and equipment function during milking can cause damage to the teat end. First evidence is a raised ring around the teat canal, leading to build-up of scar tissue around the teat opening – also known as hyperkeratosis (Fig. 18). This condition leads to the teat end being very rough, and often harbours mastitis-causing bacteria.

Fig. 24. Contagious ecthyma – teat Dr. M. Smith, Cornell U

Build-up in the teat, which prevents milk from being released from the udder, is called a pea. This is fibrous tissue caused by infection or trauma that will block milk in the teat. Sometimes it can be removed by a veterinarian but the damage may be permanent.

In ewes that are still nursing their lambs, there is a risk of teat damage due to teat biting (Fig. 23). This can cause lesions which could be very painful to the ewe during milking, and can potentially harbour contagious pathogens. Nursing ewes may develop contagious ecthyma (orf, soremouth) on the teats, picked up from the lambs (Fig. 24).

5.2 EXAMINATION OF THE MILK OF AN INDIVIDUAL SHEEP

5.2.1 PHYSICAL INSPECTION OF MILK

APPEARANCE OF NORMAL MILK

Normal milk should be white in colour, but may range to a white-yellow. Milk should have a thin consistency, with no solid milk clots whatsoever.

APPEARANCE OF COLOSTRUM

Colostrum has a thicker consistency than regular lactation milk, and is generally yellow in colour. Colostrum in sheep may be very thick and yellow – caramel-like, more so than in cattle or goats. If no clots are present this is not abnormal. This appearance only lasts a few days after lambing, then the milk returns to its normal colour and consistency.

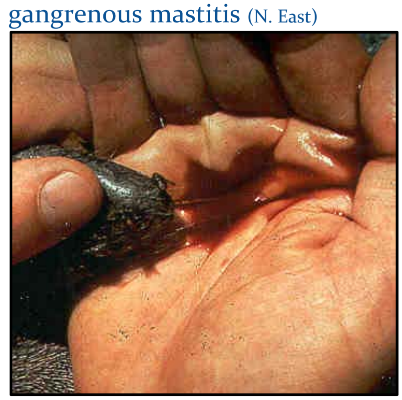
Although colostrum may be considered 'normal' from a physiological aspect, it is not considered normal in terms of marketing (for human consumption). Ontario regulations consider colostrum to be 'abnormal' and therefore require that it not be mixed with milk in the bulk tank (milk for market). For cattle and goats the Ontario Provincial Milk Act describes abnormal milk as that which

a) comes from an animal 15 days prior to and 3 days after parturition, (or longer if it still contains colostrum)
b) contains blood or other foreign particles;
c) is watery or coagulated;
d) has odours that adversely affect its organoleptic characteristics;
e) is contaminated by chemical, toxin, drug or any other foreign substance.

APPEARANCE OF ABNORMAL MILK

The characterization of abnormal milk can be quite subtle, from faint flakes in otherwise normal milk, to paste-like clots with no liquid present. The colour can range from white to white-yellow. In some cases, if there is an acute infection such as *E. coli*, milk is of thin consistency with little-to-no clots, but the liquid is yellow and clear. With severe gangrenous mastitis, the milk may look like red serum – with or without clots of milk (Fig. 25).

Fig. 25. Secretion with gangrenous mastitis (N. East)



Sometimes, there may be a variation in the milk with the addition of fresh-appearing blood. This is due to trauma of the udder, however is not by infection, but from injury. This is quite common in early lactation animals after a difficult lambing, but can be caused by any injury to the udder.

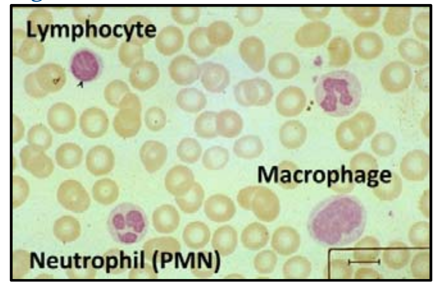
5.3 DETECTION OF INFLAMMATORY SOMATIC CELLS IN AN INDIVIDUAL SHEEP

5.3.1 WHAT ARE SOMATIC CELLS?

Somatic cells, mostly comprised of white blood cells are defense cells of the immune system that are excreted into milk to kill bacterial infections in the udder. They also include cells sloughed from the alveoli (Section I.1.1). Somatic cells are always found in milk in low numbers; however, the number and type of cells varies greatly when there is infection in the udder. The following are cells that are found in normal milk:

- Macrophages (big eaters in Latin); Approximately 80% of all cells in normal milk
- Lymphocytes; Approximately 15% of all cells in normal milk
- Polymorphonuclear leucocytes (PMNs) (also called neutrophils); <5% of all cells in normal milk
- Epithielial duct cells; <5% of all cells in normal milk

Fig. 26. White blood cells



The primary role of macrophages and lymphocytes are to act as an alarm system in the udder, and signal when there are bacteria present in that gland. When this occurs, there is a huge influx of PMNs from the blood stream, and these cells destroy the bacteria in the tissue and the milk alveoli in the gland (see Section I for normal anatomy). At this time, the concentration of cells in the milk changes, and PMNs are the dominant cells found in the milk, until the infection subsides.

5.3.2 SOMATIC CELL COUNT (SCC)

Somatic cell count (SCC) is a diagnostic measurement to determine the approximate level infection in the udder. As previously mentioned, there are always somatic cells present in the milk, so there is no time that SCC will ever be zero. If SCC of a sample of the gland is below a particular threshold, it is assuming that the gland is healthy.

When an infection occurs, the influx of PMNs into the gland will trigger an increase in SCC, providing a good indication to producers that there is an infection in the gland. This is especially important for subclinical cases of mastitis, where there are no visible changes in the milk to indicate an infection, but the increase in SCC can make producers aware of a possible infection in the gland.

SCCs are measured in the amount of somatic cells that are found in one mL of milk. The unit of measurement is cells/mL of milk. A simplified way to understand these SCC is a linear score, which is a mathematical conversion of SCC. This value is easy to interpret both on an individual ewe level and flock level, and can be easily correlated with milk loss. Table II.3 is a list of linear scores, and its corresponding SCC value. Each doubling of the SCC results in an increase in linear score of 1. It also includes ranges for values for which it is more likely the udder is healthy and values for which it is more likely mastitis is present.

Table II.3. Relationship between linear score and somatic cell counts

LINEAR SCORE	SOMATIC CELL COUNT (CELLS/ML)	
0	12,500	Healthy Udder
1	25,000	
2	50,000	
3	100,000	
4	200,000	
5	400,000	Mastitis
6	800,000	
7	1,600,000	
8	3,200,000	
9	6,400,000	

5.3.3 HOW ARE SOMATIC CELLS MEASURED?

SCC values can be determined at the individual gland, the individual ewe, and whole flock level. Milk should be aseptically taken into milk vials. These samples don't require refrigeration for transportation; however, it is important that they are preserved properly. Bronopol tablets are used to preserve the milk. Vials with these tablets can be purchased from a number of sources on-line or usually obtained from a laboratory that offers SCC counting. These laboratories will have automated cell counters, which detect particles in the milk and are accurate for sheep's milk. In Ontario, CanWest DHI[3] and the Agriculture and Food Laboratory, University of Guelph[4] offers this service.

Some sheep dairies may wish to invest in on-farm cell counting techniques. There are portable cell counters that can be easily used before milking to determine SCC status of a gland. A milk sample is taken aseptically, and then drawn up immediately into a plastic cassette, which is then inserted into the cell counter. A SCC value is then determined based on the milk in the cassette. This information can also be transferred to a computer for easy record keeping. A common cell counter that is available on the market is the DeLaval Cell Counter DCC.

5.3.4 WHAT IS A NORMAL SCC FOR DAIRY SHEEP?

When measuring SCC for udder health, it is important to have a benchmark SCC level, to know if a gland is "normal", or healthy. Any SCC over this benchmark can be an indication that there is an issue in the gland. Even though these benchmarks are a good guide, producers should strive for values under these levels for optimal udder health.

In dairy cattle, a normal udder health value to strive for is 200,000 cells/mL. In goats, the benchmark SCC level is significantly higher at 600,000 – 800,000 cells/mL. This benchmark increase is not surprising, as goats have a different secretory system than cows (apocrine vs. merocrine), which yields a higher SCC value, as described in Section I.1.1.

As sheep have the same secretory system as goats, it could be assumed that the benchmark SCC levels would be similar. However, research has shown that normal SCC values of sheep and goats are quite different. There is no established benchmark for sheep, however based on research – it is likely more similar to dairy cattle than to dairy goats. A flock value of < 500,000 cell/mL should be attainable, even when ewes are in late lactation. **Individual sheep values of > 400,000 (linear score ≥5) should be strongly suspected of having mastitis. Sheep can maintain an udder health SCC level < 200,000 cells/mL (linear score ≤4)** (See Table II.3).

5.3.5 WHAT LEVELS ARE EXPECTED WHEN MASTITIS IS PRESENT?

An SCC values over the benchmark of 200,000 cells/mL is suggestive of a subclinical infection, although healthy udders may have values in this range. If accompanied by clinical signs of infection, it can be determined as a clinical infection. With subclinical mastitis, values > 400,000 cells/mL are often seen. Clinical mastitis often has SCC values > 1,000,000 cells/mL. However, interpretation of SCC values should also consider other influences as detailed below.

---

[3] http://www.canwestdhi.com/ Tel: 1-800-549-4373

[4] http://www.guelphlabservices.com/AFL/raw.aspx Tel: (519) 767-6299; Toll Free: 1-877-UofG-AFL (1-877-863-4235); Fax: (519) 767-6240; E-mail: aflinfo@uoguelph.ca

5.3.6 OTHER FACTORS THAT INFLUENCE SCC LEVEL

There are a variety of factors that can affect SCC levels in ewes other than mastitis.

- Stage of lactation: increased levels are generally found in both early and late lactation.
- Age of ewes: older ewes have had longer opportunity to be exposed to udder infections from previous lactations; they generally have a higher SCC than younger animals.
- Some breeds tend to have higher levels than others.

However, most important are the factors which also predispose to mastitis as has been covered in Section II.4.

5.3.7 CALIFORNIA MASTITIS TEST (CMT)

A CMT is a practical tool that is used on-farm to detect ewes that have increased SCC. This method of monitoring udder health is simple, and producers can get almost instantaneous results, and allows them to pinpoint their high SCC animals, and those with subclinical infections in a very easy manner.

HOW DOES A CMT WORK?

A CMT is a system that uses a paddle split into four wells to test individual glands for SCC level in an animal (Fig. 27). Initially designed for dairy cattle, it can be easily used with sheep, by just using two of the wells. Milk is mixed with a CMT reagent (purple solution). If somatic cells are present a thickening or gelling of the milk will occur. The CMT reagent reacts with the nucleic acid of the somatic cells present in the milk. The higher the SCC, the more gelling that occurs. These reactions are categorized into five categories, from negative to a strong positive test. (Table II.4).

Fig. 27. CMT tools

The CMT reagent also indicates how acidic or alkaline (pH) the milk is. Normal milk is slightly acidic (pH 6.6 to 6.8). As SCC climbs, the milk becomes more alkaline and the reaction appears more intensely purple.

Fig. 28. Strip cup to inspect milk

One of the main benefits of the CMT is that the reagents will not react with other substances such as blood or manure. Having said that, it is important to make sure that the paddle is as clean as, as excess debris could affect how to milk and reagent solution moves within the quadrants of the paddle.

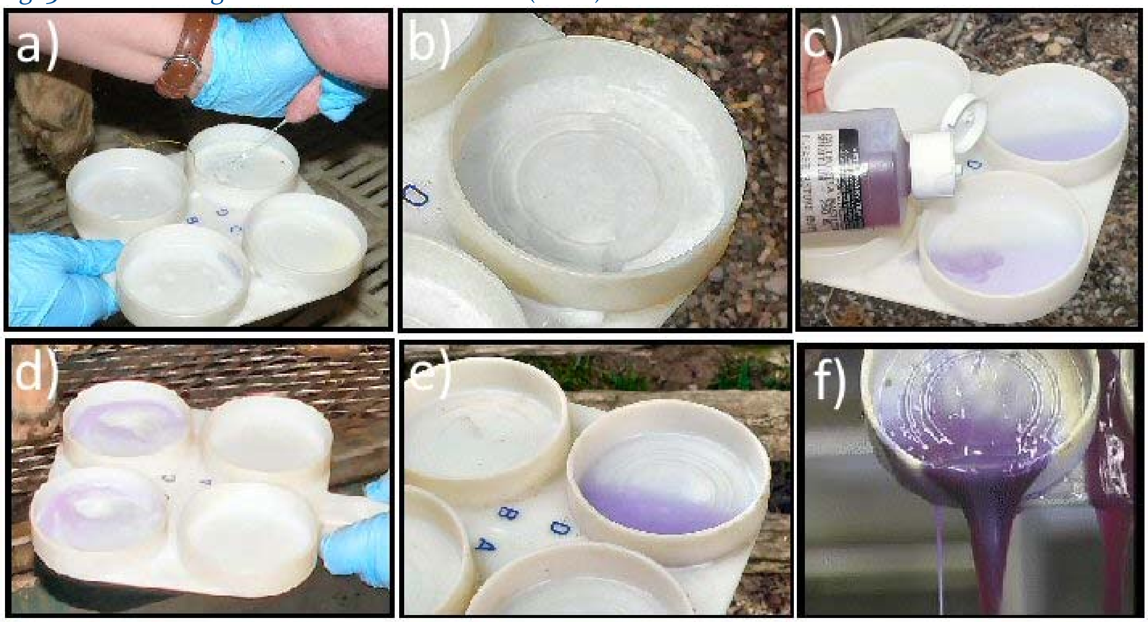
HOW TO PERFORM A CMT

- Gloves should be worn at all times
- The udder and teats should be clear of dirt before sampling
- The foremilk should be removed prior to testing (Fig. 28).
- Use a strip cup to allow visualization of the milk against a black background. If the milk appears abnormal with clots and discolouration, mastitis is present and the CMT is not necessary to perform (Fig. 29).

Fig. 29. Clinical mastitis

- Place the paddle underneath the ewe, and sample milk into one well per gland (Fig. 30a).
- The paddle can be held in any direction, but be consistent so it is clear which gland corresponds to each well.
- At least 5 mL (1 teaspoon) should be expressed into each well.

Fig. 30. Performing a California Mastitis Test (CMT)



- Pour out excess milk by tilting the paddle, ensuring that there is enough milk covering the complete area of the quadrant (Fig. 30b).
- Keeping the paddle tipped, add an equal amount of reagent to the milk (i.e. 5 mL which is about a teaspoon), making sure that the total amount of liquid does fill more than half of the well (Fig. 30c).
- Bringing the paddle back to a level position, mix the milk and reagent together by moving the paddle gently in a circular motion, for approximately 10 – 30 sec (Fig. 30d)
- Observations of the consistency of milk can be done while swirling, to see if any gel is forming. Alternatively, the paddle can be tilted to observe the consistency of the liquid as it is pouring out (Fig. 30e and f).

USING THE RESULTS OF A CMT

The CMT can be used for daily monitoring of udder health on-farm (Fig. 31). If you suspect that a ewe may have an udder infection, the milk can be tested immediately. If the reaction is ≥ CMT 1+, a milk sample should be cultured. CMT is a good screening process to ensure that only glands that are high in SCC levels are cultured, which decreases the cost.

However, it is important to understand that this technique does have its limitations. Interpretation of the score is subjective. False positives can occur in ewes that are early or late in lactation. SCC is a more reliable test than CMT. Table II.4 is adapted from the Canadian Bovine Mastitis Research Network TACTIC Udder Health Veterinary Kit, and describes the visual identification of the range of CMT scores.

Fig. 31. CMT reaction

Table II.4. California Mastitis Test scoring system

SCORE	INTERPRETATION	VISUAL CHARACTERISTICS OF LIQUID	SCC RANGE (CELLS/ML)
N	Negative Sample (Fig. 31 #1)	The mixture does not change, and remains the same liquid consistency of milk with bluish/purple tinges.	0 – 200,000
T	Trace Sample	The mixture will thicken slightly like very thin porridge, however, it can revert back to its original state when moving the paddle.	150,000 – 500,000
1	Weak, but Positive Sample (Fig. 31 #2)	There is slight thickening of the milk like thin porridge; no gel forms; when swirled, the mixture will climb the walls of the well; and when poured out, the mixture flows a steady pace.	400,000 – 1,500,000
2	Distinctly Positive Sample	Gel is beginning to form; when swirled the gel tends to clump in the middle of the well; when poured out, the gel will pour out first, leaving some liquid is remaining in the well.	800,000 – 5,000,000
3	Strongly Positive Sample (Fig. 31 #3 and #3a)	The entire mixture is gel; when swirled it clumps in the middle; and when poured out of the paddle, no liquid remains in the well.	> 5,000,000

5.4 DETECTION OF UDDER PATHOGENS IN THE INDIVIDUAL SHEEP

Commonly, detection of pathogens in the udder involves culturing of the milk for presence of bacteria and mycoplasma. It is unusual that we choose to detect viruses from milk although tests have been developed for detection of maedi visna virus using a polymerase chain reaction (PCR), used to detect the DNA of the virus.

5.4.1 OBTAINING AN ASEPTIC MILK SAMPLE FROM AN INDIVIDUAL EWE FOR CULTURE

When taking a milk sample for submission for culture, it is important that preparation of the teats is done correctly to ensure that samples do not become contaminated. Ideally, these samples would be taken at the time of milking, as udder preparation and disinfection are being done at that time and milk let-down is maximized, which helps with manual teat sampling.

MATERIALS REQUIRED

The following materials are required for aseptic milk sampling on sheep flocks:

- Sterile milk vials; ideally a snap-cap vial, which is easier to open, as compared to a twist top vial (Fig. 32)
- Gloves
- Labels and markers for labelling the vials
- Udder wash solution and cloths/towels to initially disinfect and dry the udder and teat
- Sterile swabs that are soaked with 70% isopropyl alcohol to disinfect the teat
- Cooler with ice packs to put milk samples in when transporting from the farm

Fig. 32. Materials for milk culture

PREPARING THE UDDER AND TEAT

- Before doing any type of udder and teat preparation, it is important to remove any excess dirt or manure from the udder, as it could fall into the milk vial during milking, and contaminate the sample.
- Gloves should be worn at all times during sampling
- The udder and teats should be clean and dry, using udder wash or wipes and a single service cloth or towel (Fig. 33).
- Four or five strips of milk should be extracted from the teat before taking the sample, as the milk closest to the teat end has a chance of having increased pathogen loads.
- Teats should be fully disinfected with sterile swabs.
- Teat ends should be thoroughly wiped with a new sterile swab, which should kill the majority of pathogens on the exterior of the teat. Use new swabs until the swab is clean (Fig. 34).

Fig. 33. Clean the udder

Fig. 34. Clean the teat

TAKING THE SAMPLE

- All sampling should be done in the same fashion as stripping foremilk during udder preparation or hand milking, by manually stripping the teat in a downward motion (Fig. 35).
- When removing the cap of the milk vial, it is essential that the lid is held with the inside of the cap downwards, to prevent any debris from getting inside.
- The vial itself should be held at a horizontal angle to ensure that no debris will get into the vial.
- Ensuring that the teat end does not come in contact with the tube, begin stripping the milk into the tube.
- Only fill the milk vial up to approximately ¾ of the full volume, as there is a chance of the vials to open when frozen if they are too full.

Fig. 35. Take a sterile milk sample

- If composite samples are being taken instead of individual gland samples, ensure that equal amounts of milk are taken from each gland.
- Teats should then be disinfected after sampling with post-milking teat dip.

HANDLING OF SAMPLE INCLUDING STORAGE AND SHIPPING TO LABORATORY

- It is important to properly label the milk samples to make sure that each sample is identified properly (Fig. 36). The following information should be included on the label:
  - Date
  - Farm name
  - Ewe unique identification number
  - Gland sampled (left or right)
  - Reason for sampling (i.e. mastitis case, high SCC, positive CMT reaction)
- Samples should be placed in a cooler with frozen ice packs, and sent to a lab as soon as possible. If samples are being held at the farm for an extended period of time, they should be frozen immediately.
- For submitting samples, it is important the flock veterinarian fill out the appropriate paperwork for laboratory submission.
- If milk samples are stored in a 4 °C fridge, they should remain there no longer than 24 h before culturing.
- If the sample is frozen in a -20 °C freezer (normal temperature of a home freezer), it should remain there no longer than 1 month before submitting for culture.
- If testing for mycoplasma rather than bacterial pathogens, it is essential that the milk samples remain unfrozen but they must be processed by the laboratory within 72 h of taking the sample.

Fig. 36. Labelled for submission

Fig. 37. Culturing milk on blood agar plate at the laboratory

5.4.2 HOW TO INTERPRET MILK CULTURE RESULTS

Results from the lab give information of what bacteria are found in each sample, and the number of each type of bacteria that are found on the milk culture. Generally we can distinguish culture results into three categories:

- Culture positive
- No growth
- Contaminated

CULTURE POSITIVE RESULT

One bacterial type (e.g. colony type) is prevalent on the plate, and is assumed to be the cause of the mastitis infection. If the plate has two different types of bacteria present, one being an important pathogen, it is considered the source for the mastitis infection.

Fig. 38. Positive culture results

Source: http://www.healthhype.com/

NO GROWTH RESULT

Even with samples from cases of clinical mastitis, there are times that the samples will show no growth on the plates, despite there being an obvious infection. There are a variety of reasons for this result, including:

- Inadequate volume of milk submitted for culture
- Non-bacterial infections, such as viruses (e.g. maedi visna), yeast or mycoplasma
- Acute and systemic infections that have already been cleared by the body at the time of milk sampling, such as *E. coli* infections. The SCC levels may still be very high but the bacterial infection has been cured
- The ewe has been recently treated with antibiotics and their presence in the milk is stopping the growth of bacteria on the plate
- Not drying the teats well enough and contaminating the milk with a disinfectant

CONTAMINATED RESULT

Contaminated samples are generally defined as three or more bacterial species or colony types on a culture plate from a milk sample. There are usually three or more different types of bacteria. Some of these bacteria could be found in the milk, but some could be from debris that fell into the vial while taking the sample, dirt still remaining on the teat end or from dirty hands recontaminating the teat and/or lid of the vial.

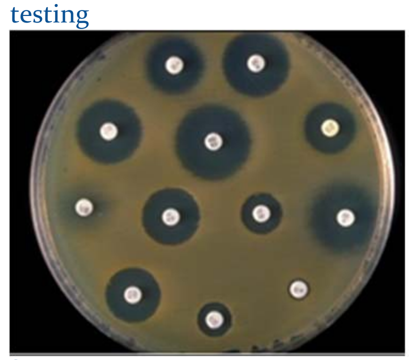
NUMBER OF BACTERIA / COLONY FORMING UNITS

The number of **colony forming units** per millilitre of milk (CFU/mL) that are reported by the laboratory is also important when interpreting results. In Fig. 38, each “dot” on the plate is one CFU. The CFU/mL is reported as the number of bacterial colonies that grow on culture plates, multiplied by 100 to account for volume of the sample that was actually plated for culture. For example, if there are two colonies on a culture plate, this result is reported as 200 CFU/mL. In general, if there are 1,000 CFU/mL or greater (i.e. > 10 bacterial colonies on the plate) for a single bacterial type, it can be assumed that that gland is infected with that bacteria, however, some research suggests that a CFU cut-point can be anything over 200 CFU/mL.

5.4.3 ANTIBIOTIC SENSITIVITY TESTING

Antimicrobial sensitivity testing is a procedure done to determine whether or not mastitis-causing bacteria are sensitive or resistant to a specific antibiotic (e.g. penicillin, tetracycline, etc.).

Fig. 39. Antibiotic sensitivity testing



Source: http://textbookofbacteriology.net/index.html

To do this test, a broth mixture containing the bacteria previously isolated from the milk sample, is poured over an agar plate. Small paper disks the size of a pencil end that have specific antibiotics added to them are placed on the plate. The antibiotic leaches out into the agar around the disk. Bacteria will grow in the agar where there are no antibiotics to retard their growth but not grow around the disks where the antibiotic is present – unless that bacteria are resistant to that antibiotic in which case they will grow right up to the disk. So a clear zone around the disk is good news – that antibiotic may be effective. No zone around the disk is bad news, that bacterial isolate is resistant to the antibiotic. The lab report will come back that the bacteria is either sensitive (S), resistant (R), or is moderate sensitive (I) to that antibiotic depending on how close they grow to the disk containing the antibiotic. This information can help when selecting the correct treatment for that case of mastitis.

5.4.4 ROUTINE FLOCK CULTURE

WHOLE FLOCK CULTURE

Routine culturing of lactating ewes will improve understanding udder health in a flock. Culturing milk samples from all lactating ewes at once (whole flock culture) may be recommended for flocks in which SCC levels at the flock level (e.g. bulk tank) are markedly elevated or when there is a high incidence of clinical mastitis. In this case, culturing the entire lactating flock can identify problem animals. Whole flock cultures are done initially on composite samples (both glands in the same vial), and will identify ewes that may need to be treated, culled or identified and milked last.

TARGETED CULTURE OF INDIVIDUAL EWES

Certain animals or certain times of lactation may be targeted for culturing as part of a program to screen for mastitis cases. This routine sampling can be done when whole flock culturing is not required. Some key screening criteria to culture animals are as follows:

- Ewes with clinical mastitis
- Ewes with abnormally high SCC's (e.g. ewes > 400,000 cells/mL or ewes > 200,000 cells/mL if mastitis levels are low (Section VII))
- Ewes with CMT reactions 1+ or higher
- Ewes at lambing or at dry-off
- Purchased ewes

6. DETECTION OF MASTITIS USING POOLED MILK SAMPLES

Bulk tank or pooled milk can be used as an inexpensive screening test to detect prevalent pathogens in a flock. The main pathogen that is relevant in these samples is *Staph. aureus* in ewes. This method of detection is an ideal option for a screening program, with regular sampling (e.g. monthly) to monitor changes in bacterial populations in flocks.

6.1 CULTURING POOLED MILK

6.1.1 OBTAINING AN ASEPTIC MILK SAMPLE FROM THE BULK TANK OR BUCKET

Fig. 40. Bucket sample

Bulk tank samples are a good way to monitor for some mastitis pathogens in the flock. The best time to take a sample is just before the milk truck arrives so that the maximum number of milkings is represented. When sampling from the bulk tank, it is important that the milk is agitated properly before sampling to ensure that all milk is mixed properly to get a truly representative sample for the flock. Agitation should occur for approximately five minutes before sampling.

Milk samples should be taken from the top of the tank from the manhole. The outlet at the bottom of the tank should not be used for sampling, as there can be bacterial build-up in this pipe, thus giving a biased milk sample. A clean sanitized dipper should be used for collecting milk. After collection, milk is aseptically placed in a sterile milk vial (by pouring or use of a sanitary straw), as used for individual ewe culture, and refrigerated or frozen until it can be cultured as outline in Section II.5.4.

Fig. 41. Pooled sample

When milking into buckets, which are to be frozen for later delivery to the processor, a sample should be taken from each bucket (Fig. 40) and then pooled in a single vial (Fig. 41). Prior to sampling, each bucket should be thoroughly mixed and sampled using a clean, sanitized dipper. This sample only represents one milking but is still a good way to monitor mastitis pathogens in the flock.

6.1.2 INTERPRETING RESULTS

With pooled milk samples, there is often an environmental pathogen cultured (e.g. coliforms, *Pseudomonas*). There is a chance that this group of pathogens is transmitted directly to the bulk tank from mastitis infections, however, it is also probable that these pathogens are from environment. This environmental population can be found anywhere from wet and dirty udders during milk preparation to faulty equipment in the parlour. These bacteria if present in high numbers may indicate a severe hygiene problem in the milking system. Please see Section V Quality Milk, for more information.

Any contagious pathogens that are present in a high enough prevalence should be detected in the bulk tank sample. For example, a pooled sample, which is positive for *Staph. aureus*, strongly indicates that there are several ewes in the flock infected with this important contagious pathogen. See Section VI.4 for more information on how to manage this.

6.1.3 INTERPRETATION OF RESULTS FROM POOLED SAMPLES

Culture results from pooled milk can be reliable; however, they do not have the capacity to detect pathogens as efficiently as culturing individual ewes. If there is a high prevalence of a particular pathogen, specifically *Staph. aureus*, pooled milk culturing should be able to detect it in the sample. However, if there is a low prevalence of a major pathogen, the culture results could give a false negative result, where some ewes are infected but too few to be picked up on a pooled sample.