Mastipedia

Move to vaccination

HIPRA

The Reference in Prevention for Animal Health

In the prevention of mastitis, **1+1=3**

Mastitis is the most costly disease of dairy cattle and each year causes economic losses of 35 billion dollars in the global dairy industry, which represents a 7-8% shortfall in dairy revenue.

In the face of such losses the implementation of mastitis control programs should be a priority for the cattle industry today. Effective control programs are based on detailed assessment of all available data and implemented with a holistic approach taking into account both the animal's environment (infection pressure), and the animal itself (ability to resist infection). As recently recalled by Dr Ynte Schukken at the NMC conference held in Ghent in 2014, in the prevention of mastitis and production of quality milk, one and one make three (1+1=3). Which means; the synergy of adding together good management (1) and an excellent vaccination plan (1) gives the best results (3).

In order to help the veterinary professional in this task, HIPRA has developed this information pack, entitled MASTIPEDIA. In it, you will find a review of the basic concepts of a milk quality programme from immunology to the milking parlour, covering the environment and milking routine on the way. Not forgetting key aspects of a control programme such as monitoring and appropriate communication.

HIPRA remains committed to the prevention of mastitis and to the quality of milk, by providing the professionals not only with innovative vaccines, but also with the knowledge to use them. After all, in prevention, one and one makes three.

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Introduction

ANATOMY AND PHYSIOLOGY OF THE UDDER

The udder is composed of 4 anatomically separate mammary glands, or quarters, divided left from right by the suspensory ligament and anterior from posterior by a thin membrane.

The milk is produced in the alveoli and is transported through a duct system to the cisterns (udder and teat) where it is stored before finally being evacuated during the milking. 70% of the milk is stored in the alveoli, while the other 30% remains in the udder cistern.

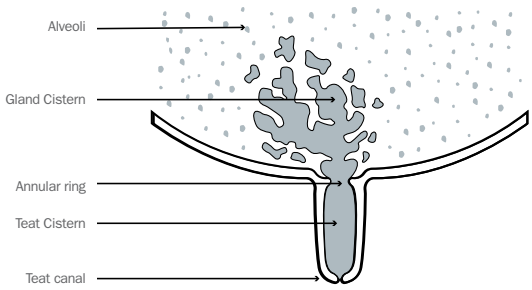

Figure 1. Anatomy of the udder.

Each alveolus is fed by an arteriole which contains blood with all the elements needed for milk production. The cells that cover the alveolus segregate milk, transforming the elements from the blood into milk components (lactose, protein, fat). To produce 1 litre of milk, 500 litres of blood must pass through the udder.

Surrounding each alveolus are the myoepithelial cells, which are tiny muscles that squeeze the alveolus to let the milk out during milking. This is called milk let-down and is stimulated physically (preparation of the udder before attaching the milking machine) and by the environment (cows waiting to be milked), activating a whole series of hormonal events. This stimulation sends signals to the brain to produce oxytocin. The oxytocin goes through the circulatory system and takes around 1-2 minutes to reach the udder. Negative stimuli (stress in the cows) causes the release of adrenaline, which contracts the blood vessels and reduces the effect of the oxytocin.

An intramammary infection (IMI) can cause permanent damage in the secretory tissue, which can be replaced by scar tissue, decreasing milk production.

Natural mechanism against mastitis

The teat cistern and the gland cistern are connected by the annular ring. The perfect teat should be smooth and flexible. The **teat canal** is surrounded by muscle in the form of a sphincter which has the function of closing teat canal.

Genetic selection for higher yielding and faster milking cows to improve the efficiency of milk harvesting has resulted in an increased risk of new intramammary infections. Higher yielding cows tend to be “faster milking” with higher peak flow rates, in part due to a more open teat canal, resulting in increased infection risk compared to lower yielding “harder to milk” lower peak flow rate cows. The internal part of the teat canal is covered by **keratin**. Any bacteria present in the teat canal tend to be trapped in this keratin and both are removed during the milking process. The keratin is replaced and in a well milked cow the production and removal are in balance. A poorly set up or badly maintained milking machine can upset this balance resulting in excessive keratin production and removal a condition known as hyperkeratosis.

Udder conformation is critical for ease of milking. The teat end is an important first line defence against new intramammary infections and if damaged not only can this defence be compromised but the teat end may then harbour mastitis pathogens such as *S. aureus*.

MILK COMPOSITION: QUALITY PARAMETERS

The value of milk produced on a dairy farm is based on both quality and composition. In order to maximise milk price the following parameters need to be optimised.

a. Physical and chemical composition

Milk is composed of water (84-89 g/100 ml), fat (2.6-8.4 g/ml), protein (2.4-6.5 g/100 ml), lactose (2.4-6.1 g/100 ml) and ash (0.6-0.9 g/100 ml). Industry and consumers demand high quality milk.

For milk contracts where milk is to be processed for example into cheese or butter a high concentration of fat or protein (specifically casein in the production of cheese) will add value. Other contracts focus on volume where litres of production are more important than the concentration of fat or protein.

b. Somatic cell count (SCC)

Somatic cells are leukocytes (99%) plus epithelial cells. When an infection occurs, leukocytes arrive at the infected zone in massive numbers. This leads to an increase in SCC in milk,

which indicates that there is an inflammatory reaction occurring. Cows over 200,000 cells/ml are considered as infected (100,000 cells/ml for heifers). However, there are some exceptions. For example, a cow with a SCC of 50,000 might be infected and another with 300,000 may be healthy. This can occur when mastitis is caused by *S. aureus* - we may see a low SCC (big variations) but bacteriologically, the cow is infected. A sudden increase in the SCC frequently indicates a new infection, while a continuous increase is normally a sign of chronic infection. Bulk-tank somatic cell count (BTSCC) or herd SCC gives an indication of prevalence or proportion of infected cows within a herd. As a rule of thumb for every 100,000 cells per ml increase in BTSCC there is an 10% increase in the proportion of infected cows within the herd. BTSCC targets will vary from country to country and herd to herd however a 400,000 cells/ml upper legal limit for milk to be used for human consumption is common in many countries.

c. Bacteriology

Milk is a very good substrate for bacteria and for sanitary reasons, levels of bacteria (colony forming units – CFUs) must be controlled. We can classify the infection according to the parameters below.

	LOW	MODERATE	HIGH	VERY HIGH
Str. agalactiae	< 5 CFU/ML	5-50 CFU/ML	50-100 CFU/ML	> 100 CFU/ML
S. aureus	< 50 CFU/ML	50-200 CFU/ML	200-500 CFU/ML	> 500 CFU/ML
Str. not agalactiae	500-700 CFU/ML	700-1200 CFU/ML	1200-2000 CFU/ML	> 2000 CFU/ML
Coliforms	< 100 CFU/ML	100-400 CFU/ML	400-700 CFU/ML	> 700 CFU/ML
CNS	< 300 CFU/ML	300-500 CFU/ML	500-750 CFU/ML	> 750 CFU/ML

To be collected, milk from the bulk tank has to be under 100,000 CFU/ml by law in all European countries.

CFU and BTSCC thresholds are determined from a 3 month geometrical mean (the n^{th} root of the product of n numbers). With these parameters, mainly with the somatic cell count, we can calculate the sanitary index. To have a healthy herd, it's a good idea to at least do one individual cow SCC recording every month.

MICROORGANISM AND EPIDEMIOLOGY

In cow's milk virus, yeast and bacteria can be found. In mastitis, the most important pathogens are bacteria.

Bacteria are normally classified into 3 groups: environmental, contagious and secondary pathogens. Even though this classification is the most commonly used, it is not very precise, because we know that some can act in different ways depending on the situation.

a. Contagious

Contagious organisms are well adapted to survival and growth in the mammary gland and are frequently responsible for chronic infections. Their prevalence increases with days in milk. **Transmission takes place during milking** and **biosecurity** management helps to avoid the entry of these pathogens. When new animals arrive on the farm, they should be quarantined until the results confirming negativity arrive.

Staphylococcus aureus
- Highly pervasive and chronic.
- Feeding waste milk, cross-sucking and flies are other risk factors that have to be considered even if the transmission normally takes place during milking.
- *S. aureus* and other staphylococcal bacteria have the ability to produce Biofilm.

Streptococcus agalactiae
- It is a very contagious pathogen; it can only live a few hours outside the udder. Easy to cure, but requires a special treatment protocol.
- Very high SCC is found and high bulk-tank bacteriological count is due to the high excretion.

Mycoplasma spp
- When this infection occurs, other signs appear in addition to the mastitis: abortions, infertility, arthritis, respiratory problems.
- More than one teat is usually affected and there is no cure but slaughter.

b. Environmental

They live in the environment, mainly in the bedding, and **the infection happens between milkings.** They do not have tropism for the alveolar cells so they replicate really quickly to survive. They produce short duration intramammary infections and they have high clinical incidence when animals are immunosuppressed (after calving for example). Some, such as Klebsiella, have also shown contagious behaviour.

Escherichia coli
- To control this type of bacteria, the environment must be dry, clean and comfortable.
  The beginning and the end of the dry-off period are the highest risk periods (A. Bradley, 2004). Immunity must be reinforced to prevent this type of mastitis.

Other coliforms
- *Serratia spp* → from contaminated teat dips or water.
- *Klebsiella spp* → associated with manure and sawdust bedding. In addition cow hygiene scores are correlated to the degree of Klebsiella contamination.

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Streptococcus uberis

- 30% of the infections during the last third of the dry-off period.
- It is found in manure and organic matter, including bedding (often in straw).
- To prevent it, the milking routine is very important, especially pre-dipping.
- Some strains can become persistent and have the potential to behave in a contagious manner.

c. Pathogens with mixed characteristics

These pathogens can have contagious and environmental transmission characteristics:

Coagulase Negative Staphylococci (CNS)

- Higher prevalence in heifers just after calving. They live in the environment and in the teat of the cows.
- Group complicated to classify.
- Some species can produce biofilm.

Streptococcus dysgalactiae

- It is commonly triggered by an alteration in the pulse of the milking machine which produces teat damage, where *S. dysgalactiae* will take up residence.
- *S. dysgalactiae* infection may evolve to a *S. aureus* infection.

d. Other pathogens that may produce mastitis occasionally

Some pathogens are less common but when present, they cause mastitis:

- *Prototheca*
- *Nocardia*
- *Pasteurella*
- *Yeast*
- *Pseudomonas*

Aetiological groups	Reservoir	Infection
S. aureus	Infected udder	During milking
S. agalactiae	Infected udder	During milking
A. pyogenes	Udder + bedding	Flies
S. uberis	Bedding	Udder preparation + Between milkings
Enterobacteriaceae	Bedding	Udder preparation + Between milkings
Pseudomonas	Water	Between milkings
Prototheca	Water + intramammary tube	Treatment
Serratia	Beds	Between milkings + teat dipping
Yeast	Environment	Treatment
Bacillus cereus	Mud	Between milkings
Mycoplasma	Infected udder	During milking
Corynebacterium	Infected udder	Poor disinfection

DETECTION METHODS

a. Clinical mastitis

The best approach is to have a mastitis register sheet to keep a record of the number of cases, treatments, duration, severity, etc., on the farm (the form referred to in sanitary index).

Detection of clinical mastitis is based on 3 parameters. Depending on how many are affected, we will have a different grade of mastitis. The classification is as follows:

Mild	Milk secretion altered	GRADE 1
Moderate	Milk secretion and mammary gland altered	GRADE 2
Severe	Secretion, mammary gland and general state of health altered	GRADE 3

To collect the data from clinical mastitis, we recommend using a form to record all of the information:

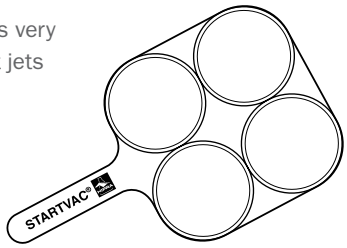
Total cases: percentage of mastitis cases in one year. The goal is to be under 40%.
Repeated cases: percentage of the total which are not first cases <20%.
Clinical mastitis in the first 30 days of lactation: percentage of animals showing clinical mastitis in the first 30 days of lactation (should be under 9%).
Severity: Proportion of severe cases (udder or cow affected) out of all the cases. The goal is to be below 20%.

b. Subclinical mastitis

California Mastitis Test (CMT)

This is a subjective test which allows us to estimate the quantity of somatic cells in a cow in each quarter. It should be done every two weeks if there is no monthly individual control on the farm, and in every animal at the beginning or end of lactation. The person who does the CMT must consider the following standards:

- Stripping before sampling is very important because the first jets can give false positives.
- CMT has to be done at the beginning of the milking because it is easier to identify due to the quantity of pathogens.



The table below shows the relationship between the CMT result and its equivalence according to the SCC.

Relationship between CMT and SCC

CMT Result	SCC x 1000
Negative	100
Traces	300
1	900
2	2,700
3	8,100

Electrical conductivity

This is integrated in most of the automatic milking systems and measures ions. This method is based on the difference in salt concentration between infected and non-infected quarters. The problem is that it frequently fails when SCC is between 100,000 and 400,000 cells/ml and other factors (stress, heat, etc.) may interfere and cause changes in conductivity.

SCC

· Bulk-tank somatic cell count (BTSCC)

This is like an X-ray of the herd and it advises us about the problem but does not define it either at herd or at individual level.

It is an economic parameter and is used as one of the criteria to pay for the milk according to quality. A bulk tank with a SCC over 400,000 cells/ml (geometrical average from 3 months) will not be collected by the industry in most countries.

· Individual Somatic cell count (ISCC)

Ideally, this shoud be done every month. It is an indicator of how the cow is reacting to the infection and lets us calculate the sanitary index. We consider as healthy, cows under 200,000 cells/ml. Cows above this threshold without clinical symptoms are considered subclinically infected. In heifers, we normally use 100,000 cells/ml instead of 200,000 cells/ml as threshold. The SCC is mainly altered by intrammamary infections (IMI) but variation can be the result of other factors (age, season, other diseases, etc.).

Monthly ISCC helps us to work with the sanitary index and make decisions based on changes detected and comparison of results from different testing points.

First infections: percentage of cows which have more than 200,000 cells/ml first control after calving (correct is under 15%).

New infections risk: percentage of cows >200,000 cells/ml in the present control which were <200,000 cells/ml in the previous (Goal<10%).

Chronic cows: percentage of cows with more than 200,000 cells/ml in 2 consecutive months (goal <10%).

Healthy cows: percentage of animals below 200,000 cells/ml. Normally it should be more than 80% of the cows.

Prevalence	< 20%
New infections	< 10%
Chronic cows	< 10%

c. Diagnosis methods

Sampling for microbiological analysis and PCR are the only truly diagnostic methods which allow us to identify the pathogen that is causing disease. The rest of the methods detect mastitis but do not give a real diagnosis.

Culture from bulk tank

	LOW	MODERATE	HIGH	VERY HIGH
Str. agalactiae	< 5 CFU/ML	5-50 CFU/ML	50-100 CFU/ML	> 100 CFU/ML
S. aureus	< 50 CFU/ML	50-200 CFU/ML	200-500 CFU/ML	> 500 CFU/ML
Str. not agalactiae	500-700 CFU/ML	700-1200 CFU/ML	1200-2000 CFU/ML	> 2000 CFU/ML
Coliforms	< 100 CFU/ML	100-400 CFU/ML	400-700 CFU/ML	> 700 CFU/ML
CNS	< 300 CFU/ML	300-500 CFU/ML	500-750 CFU/ML	> 750 CFU/ML

Having the bacterial culture/count of each type of germ can help in diagnosis, allowing us to identify where the problem is and what type of measures we need to apply. The classification of the infection is shown in the above table. However it must be remembered that potential faecal origin bacteria such as coliforms and some Streptococci not agalactiae might not originate from an intramammary source and merely be bulk tank contaminants.

Individual culture

With this type of culture, we can identify the predominant pathogen and establish a protocol (treatment, vaccination, elimination, segregation) based on the results. The individual culture can be very useful in cows with either clinical cases or elevated SCC in order to isolate and identify the most important pathogens.

PCR

PCR-based methods are now being used increasingly in bovine mastitis diagnostics with good reliability as a complement to bacterial culture. Bacterial screen is limited to a determinate number of pathogens but will detect both live and dead bacteria, it is more sensitive and faster - the result is obtained in 4 hours.

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STARTCHECK is based on real-time PCR and samples are transported using an FTA card (no refrigeration needed). It detects 4 important pathogens: *S. aureus, CNS, E. coli, Coliforms.* Samples are taken from the bulk tank and from a mastitis pool.

CONCLUSION

Mastitis is an alteration in the normal functioning of the mammary gland. It can be caused by contagious (*S. aureus, S. agalactiae, Mycoplasma*), environmental (*E. coli, coliforms and S. uberis*) or mixed pathogens (*CNS, S. dysgalactiae)*. The infection is commonly classified according to quality parameters (composition, SCC, bacteriology) and sanitary index (these are mainly calculated from the monthly individual somatic cell count).

Clinical mastitis is detected by observing clinical signs (having a mastitis control form helps greatly) and subclinical mastitis can be detected by CMT, SCC or electrical conductivity. However, real diagnosis can only be made by microbiological culture or PCR.

ADDITIONAL READING

A Practical Look at Contagious Mastitis. NMC www.nmconline.org

A Practical Look at Environmental Mastitis. NMC www.nmconline.org

Prototheca, Yeast, and Bacillus as a Cause of Mastitis. Ruben N. Gonzalez 1996 National Mastitis Council Annual Meeting Proceedings page 82.

Udder Health. Large Herd edition . J. Hulsen, T. Lam, Y. Schukken (2013).

Environmental mastitis: know your opponent. K. Larry Smith and J.S. Hogan NMC Regional Meeting Proceedings (2008).

WHAT DO YOU KNOW ABOUT...

1. The 4 quarters are:
a. Physiologically and anatomically independent.
b. Physiologically and anatomically related.
c. Physiologically related and anatomically independent.
d. Physiologically independent and anatomically related.

2. Which of the following statements about oxytocin is false?
a. It is responsible for the contraction of the myoepithelial cells.
b. It has an immediate effect.
c. Adrenaline reduces the oxytocin effect.
d. Oxytocin release is the consequence of physical and environmental stimulation.

3. Is it possible for a cow to have a low SCC and be infected with *S. aureus*?
a. No, never. *S. aureus* infections always show a high SCC.
b. Yes, it sometimes happens.
c. Yes, but only in heifers.
d. Yes, but only in chronic cows.

4. What should we check when we find a herd with high percentage of chronic cows?
a. Prevention
b. Diagnostic
c. Treatment
d. All the above

5. Which is the highest risk period for environmental pathogens?
a. During milking.
b. When a new animal enters the farm.
c. Between milkings.
d. b and c are both correct.

6. Which of the following statements is false?
a. *S. aureus*: Infection occurs during milking.
b. *S. agalactiae*: Survives a very short time outside the udder.
c. *Mycoplasma spp:* Systemic infection, different signs apart from mastitis.
d. CNS are only located in the skin of the udder.

7. Which of the following statements is false?
a. *E. coli:* the dry period is not important.
b. Coliforms: usually related to environmental problems.
c. *S. uberis:* to control it, pre-dipping is a factor to be checked.
d. *S. dysgalactiae*: related to problems with the pulse of the milking machine.

8. With regard to mastitis detection methods, which statement is not correct?
a. CMT: always do stripping before taking samples.
b. Electrical conductivity: problems when SCC is between 100,000 and 400,000 cells/ml.
c. We consider severe mastitis if there is an alteration in milk secretion and the mammary gland is affected.
d. ISCC: alteration can be the result of other factors apart from IMI.

9. Which of the following techniques is not a mastitis diagnostic method?
a. PCR
b. CMT
c. Individual culture
d. Bulk-tank culture

10. STARTCHECK is a:
a. Culture from bulk tank.
b. Culture from mastitis pool.
c. Bulk-tank somatic cell count.
d. Real-time PCR.

Find the answers below:

1c 2b 3b 4d 5c 6d 7a 8c 9b 10d

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Immunology

IMMUNOLOGY OF THE UDDER

The immune system in the cow consists of two distinct but interactive systems; the innate and the adaptive or antibody mediated. Each of these has specific functions and response times but work in concert to protect the cow from infectious pathogens.

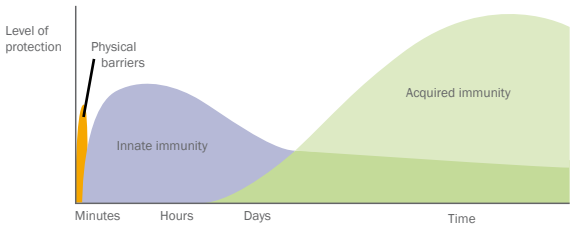

Figure 1. Adapted from Tizard 2004.

a. Innate/ natural immunity

It is present at birth and does not have to be learned through exposure to an invader. It is the predominant system in early infections. It responds very quickly and tries to eliminate the infecting pathogen. It recognizes only a limited number of antigens however; these antigens are present on many different invaders. The effect is the same no matter how many times the cow is exposed: it has no memory - it does not remember specific foreign antigens, and does not provide any ongoing protection against future infection.

Innate immunity includes following barriers:

Anatomical barrier:

Teat and teat canal. The keratin which lines the inside of the teat and traps bacteria deserves a special mention. This keratin is eliminated and replaced at each milking. The teat sphincter in another fundamental part of the anatomical barrier preventing escape of milk and prevents the entry of different pathogens[1].

Humoral barrier:

· Lactoferrin: Multifunctional protein which has the capacity to fixate iron, resulting in inhibition of iron-dependent bacteria. Higher levels are observed during involution of the mammary gland and during inflammatory processes. In ruminants, lactoferrin and IgG1 act together to inhibit *Escherichia coli* and *Klebsiella pneumonia* growth, but other bacteria such as *Streptococcus agalactiae* can actually use lactoferrin as a source of iron. In the lactation, the lactoferrin concentration is lower than that seen during involution of the bovine mammary gland which partly explain why cows in early lactation are more susceptible to intramammary infections and why mastitis is more severe in them[2].

· Complement: The complement system is made up 30 proteins that act in sequence (complement cascade). It plays an important part in the innate immunity against microorganisms through its bactericidal, opsonic*, and phlogistic functions. The amount of the complement in the milk of healthy glands of dairy cows is low but when inflammation develops and complement-dependent bactericidal, opsonic and phlogistic activities may be high in milk[3].

·Cytokines: A group of proteins that act as chemical messengers and are produced by certain white blood cells when an antigen is detected. Cytokines help to stimulate the activity of polymorphonuclear neutrophils (PMN) or inhibit the activity of the antigen. The most important cytokines in mammary infection are:

- Regulatory action*: IL-4, IL-10* and *IFN-γ*
- Pro-inflammatory action: *IL-1, IL- 6, IL-8* and *TNF-α*

*Opsonization: process whereby the surface of the antigen is marked by opsonins (immunoglubulins) so that it will be readily identified and engulfed by phagocytes for destruction.

Cellular defences:

The cells in "healthy" milk consist of macrophages, which are the predominant cell type (66- 88%), followed by PMN, lymphocytes and epithelial cells. During early lactation, the percentage of PMN tends to increase, while lymphocytes tend to decrease. In infected mammary glands, the percentage of neutrophils can reach up to 95% (neutrophils only make up 5-11% of the total cell count in normal bovine milk)[4]. In general non- infected quarters will have a lower SCC than subclinical infected ones.

· The innate immune functions on the basis of special receptors called Toll-like receptors (TLRs) that play a crucial role in "danger" recognition and the induction of immune response. Cells of the immune system recognize pathogens via TLRs. TLR stimulation via microbial products activates the innate immune response. TLRs recognize highly conserved structural motifs known as pathogen-associated microbial patterns (PAMPs), which are exclusively expressed by microbial pathogens[5].

· Monocytes and macrophages: Because they are the most abundant in non-infected mammary glands, they are considered responsible for initiating the immune response. Monocytes migrate from the blood stream to the tissues when infection occurs. There, over a period, they enlarge greatly and produce granules differentiating into macrophages. These granules contain enzymes that help to kill and digest bacteria and other foreign cells. Have phagocytic capability (phagocytosis) and an antigen presenting function. Moreover, they also secrete substances which attract other white blood cells to the site of infection (chemotaxis).

· Polymorphonuclear neutrophils (PMN): These are among the first immune cells to defend against infection. They move into the tissue by means of chemotaxis and release not only enzymes and reactive oxygen species to help kill and digest bacteria, but also substances that produce fibres in the surrounding tissue which help to trap bacteria, preventing them from spreading and making them easier to destroy. However the presence of neutrophils is like a double- edge sword. While PMN are destroying invading pathogens, they also inadvertently release chemicals which induce sloughing of secretory cells and decrease the secretory activity of mammary gland tissue. Permanent scarring will result in a permanent loss of milk production[6].

· Lymphocytes:
  · Natural killer (NK) cells: Recognize and attach to infected cells and release enzymes and other substances that damage the outer membranes of these target cells. NK cells also are capable of killing both Gram-positive and Gram-negative bacteria, and therefore could be important in preventing mammary infection[7].

b. Adaptive/ acquired immunity It starts when the pathogen survives the innate immune response (the second line of animal' s defence) and it responds more robustly to pathogens which it has previously been exposed. However it needs more time to develop after first-time exposure to a new antigen. It does have memory, however, so each time a cow is infected, the immunity response will be faster, longer and more intense. This does not mean that the immunity will last for ever.

• **Lymphocytes**
  - B → these are the responsible for antibody production and participate in the regulation of the immune system by acting as APC or interfering in the inflammatory response.
  - T
    · CD4+: helper/memory. Activation of cytokine secretion.
      - Th1 response (pro-inflammatory). More common at the beginning of lactation. Cytokines produced by Th1 cells activate macrophages and participate in the generation of Tc cells, resulting in a cell-mediated immune response.
      - Th2 response (anti-inflammatory). More common at the end of lactation and during pregnancy.

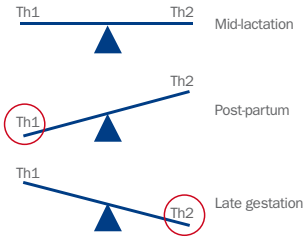

Figure 2. Adapted from Y. Schukken 2014.

    · CD8+: cytotoxic action and suppression of the activity of the immune system.

• **Immunoglobulins:** can be produced locally or systemically.
  - IgG1: is the most prevalent one in healthy mammary gland
  - IgG2: is the main one for opsonisation by PMN. Increases in cases of mastitis
  - IgA: Is found in low concentrations in milk and is predominantly present on mucosal surfaces. It seems to participate in agglutination, by preventing bacterial colonization, and in neutralization of toxins.
  - IgM: it has opsonisation function.

• **Macrophages:** act as Antigen- Presenting Cells (APC) and help the T cells to recognize invaders.

• **Cytokines**
  - Pro-inflammatory (IL-2, INF-γ)
  - Anti-inflammatory (IL-4, IL-5, IL-10)

• **Complement:** Influences the B cell response and operates at various levels; at the recruitment of B cells as APC for T cells and in the direct activation of the B cells themselves[8].

Immunology

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VACCINATION

The aim of vaccination is to increase the immunity (immunological memory) of the animal in order to prevent new infections and/or promote clearance of infection. Vaccination against bovine mastitis has an effect on acquired immunity by increasing the levels of immunoglobulins (total IgG) in blood and milk.

a. STARTVAC® is polyvalent vaccine and both antigens are described below:

***Escherichia coli* J5**

It has traditionally been considered that vaccination increases levels of IgG antibodies, but especially IgG2, which leads to enhanced opsonisation and phagocytosis.

It can also lead to serum neutralization of lipopolysaccharides due to production of anti-LPS-core-specific antigens. This is important because LPS is toxic and is released with the death of *E. coli*, which can cause toxic shock.

Vaccination with *E. coli* J5 does not prevent intramammary infections. However, the use of *E. coli* J5 reduced the severity and duration of mastitis. The primary means by which the vaccine reduces clinical severity appears to be related to opsonisation and clearance of bacteria from the gland. Duration and severity of clinical signs were positively correlated with bacterial counts in milk following intramammary challenge with either virulent or non- virulent strains.

Vaccination with *E. coli* produces short-term immunity, so booster doses are required at least every three months. This is not only refers to STARTVAC® , but common to all J5 vaccines.

The following graphs show the effect of vaccination with STARTVAC® on total IgG and IgG2 in serum and total IgG in milk after two doses (0 and 35 days) and after challenge with *E. coli* 59 days post-vaccination (14 days postpartum).

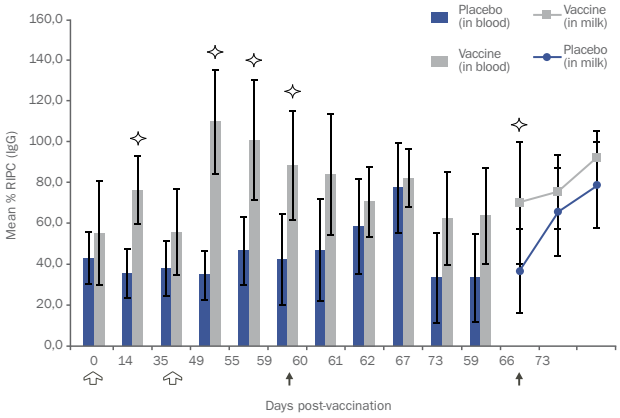

Figure 3. Anti-*E. coli* antibody response (IgG) in blood and milk after vaccination.

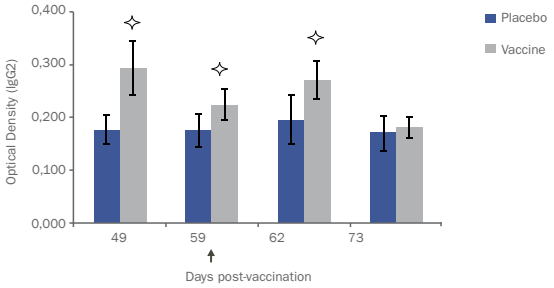

Figure 4. Anti- *E. coli* antibody response (IgG2) in blood after vaccination.

There are at least two reasons why a natural infection with *E. coli* cannot protect against a subsequent one:

- There is no cross-protection between different strains of *E. coli.*
- The endotoxin tolerance phenomenon* after an intramammary infection with *E. coli –it lasts* only 14 days.

* Tolerance phenomenon: the development of specific non-reactivity of lymphoid tissues to a particular antigen capable under other conditions of inducing immunity, resulting from previous contact with the antigen and having no effect on the response to non-cross-reacting antigens.

Staphylococcus aureus

STARTVAC® vaccination is based on increasing antibody titres against SAAC (Slime Associated Antigenic Complex). The increase of total IgG and IgG2 antibodies in serum was associated with reduced bacterial counts after experimental infection with *S. aureus.*

The following graphs show the effect of vaccination with STARTVAC® on total IgG and IgG2 in serum and in milk after two doses (0 and 35 days) and after challenge with *S. aureus* on day 59 post-vaccination (14 days postpartum).

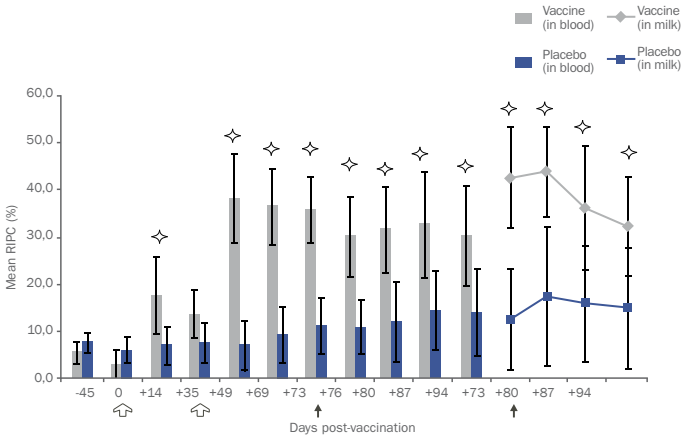

Figure 5. Anti-SAAC antibody response (IgG) in blood and milk after vaccination.

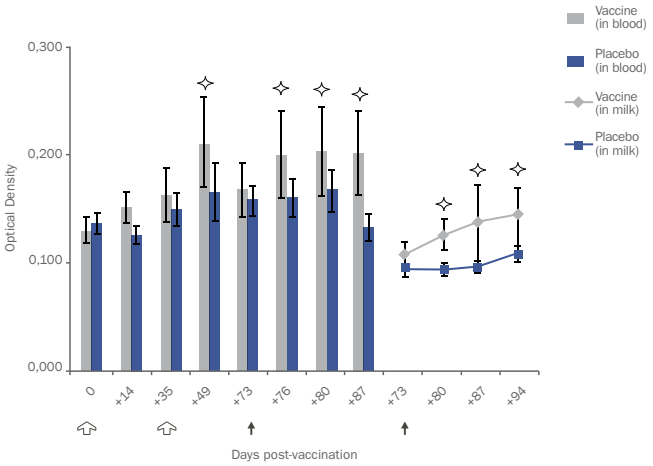

Figure 6. Anti-SAAC antibody response (IgG2) in blood and milk after vaccination.

Vaccination is associated with greater IgG2 production which facilitates the action of neutrophils. It has been shown that IgG2 develops a Th1 (pro-inflammatory) response, stimulating the acquired immune system.

When a high-biofilm-producing strain of *S. aureus* was cultivated, it was found that in-plate adhesion was decreased in the presence of antibodies (without a cellular component).

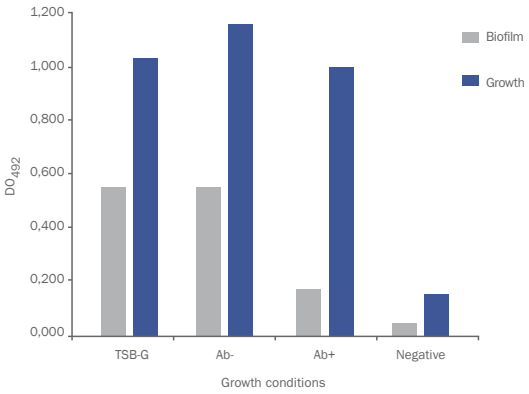

Figure 7. Biofilm assay (late adherence test) of *S. aureus* 3396.

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b. Challenge trial: Immunological response to an experimental intramammary inoculation with a killed *Staphylococcus aureus* strain in vaccinated and non-vaccinated lactating dairy cows. Sofie Piepers et al., 2014.

The aim of this study was to evaluate the effect of administration of the STARTVAC® on milk PMN concentration and viability. Secondly, the production of the antigen-specific antibodies anti-SAAC (against *S. aureus*) and anti-J5 (against *E. coli*) in blood was determined over dry period.

Results

Vaccination does not interfere in PMN numbers or viability (innate immunity), but vaccinated animals show higher levels of anti-SAAC and anti-J5 IgG (acquired immunity) in blood and in milk. Vaccinated cows seem to undergo a less severe inflammatory reaction after inoculation compared to non-vaccinated animals. This could possibly explain why no change in daily milk yield was observed in the vaccinated animals, while the non-vaccinated animals suffered from a substantial drop in milk production in the days after challenge.

Neutrophil viability

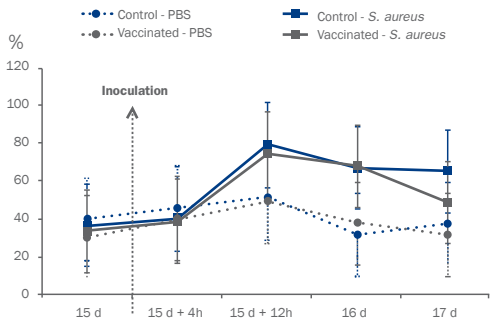

Figure 8. Neutrophil viability.

Neutrophil concentration

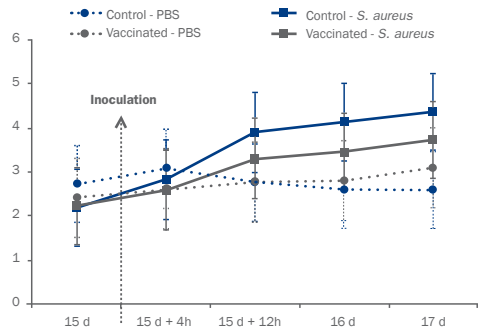

Figure 9. Neutrophil concentration.

Total IgG serum, *E. coli/S. aureus*

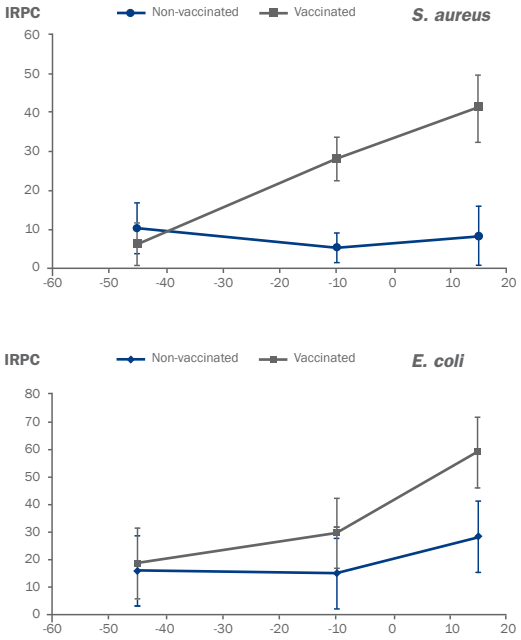

Figure 10 / 11. Total IgG serum, *E.Coli / S. aureus*.

Total IgG milk, *E. coli/S. aureus*

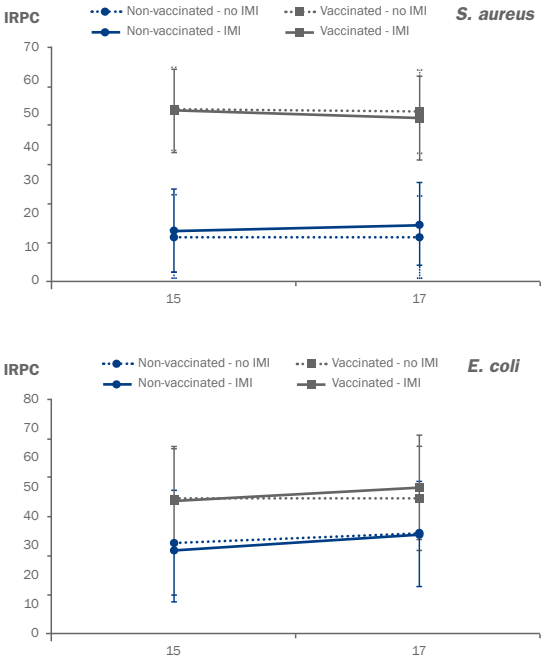

Figure 12 / 13. Total IgG milk, *E.Coli / S. aureus*.

CONCLUSION

There are many different ways to improve the natural resistance of the cow but it is worth to focus on the most important ones.

a. Innate immunity

Feeding: control of Negative Energy Balance (NEB): lipolysis produces ketone bodies (affecting the immune response).

Trace minerals and vitamins: Chelated zinc methionine, copper, vitamin E, and selenium are especially important to the proper functioning of the immune system. Supplementation with micronutrients during the dry period is very important to the immune system's response to bacterial challenge during early lactation. Current research suggests vitamin E at 1,000 IU and selenium at 0.3 ppm is optimal during the close-up dry period. Copper and zinc also appear to be important to mastitis resistance at calving. Studies with zinc methionine indicate a beneficial effect on SCC and rate of recovery when cows are exposed to bacteria. Copper supplementation before calving appears to affect the level of mastitis infections and SCC after calving.

- Selenium and vitamin E appear to have similar functions in resistance to mastitis. Vitamin E and glutathione peroxidase (an enzyme which contains selenium) protect mammary tissue from damage by free oxygen radicals produced by neutrophils and macrophages when killing bacteria. Cows receiving vitamin E and selenium supplements have fewer mastitis problems.

- Zinc: Several studies have demonstrated that supplementation of diets with zinc methionine reduces the somatic cell count (SCC). Consequently, zinc methionine may augment the killing ability of the immune cells or reduce the potency of the bacteria, thereby allowing the cow's immune system to gain a competitive edge against the bacteria. Zinc also plays a role in the maintenance of epithelial tissue and keratin production[9].

Teat end quality: If the anatomical barrier is altered, innate immunity is affected. Teat scoring is important to control the risk this represents. This can also help us to tell if there is a problem with the milking machine.

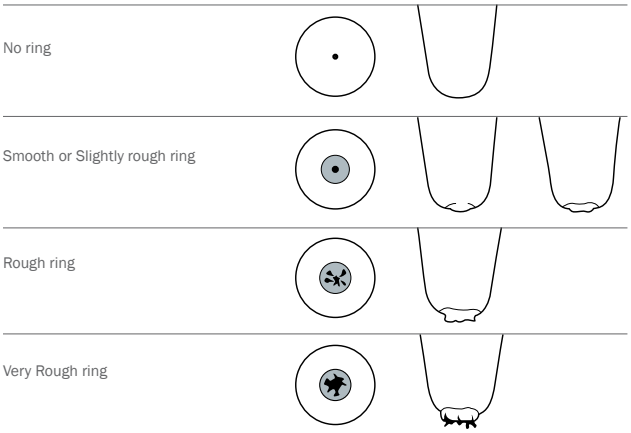

Figure 14. Teat end condition evaluation. Adapted from Mein et al, 2001.

Hygiene management: Working in clean conditions reduces the bacterial load and there will be less mastitis. This part will be explained in the corresponding chapter (Chapter 3 "Environmental control, keeping the cows clean, dry and comfortable").

Cow comfort: is also important because stress interferes with both the innate and acquired immunity of the animals (more details in chapter 3).

b. Acquired immunity

In essence, vaccination is a form of active immunization entailing the introduction of a foreign molecule, e.g. bacteria or parts of the bacteria into the cow causing the cow itself to generate immunity via the production of antibodies specifically oriented against the target. Using this binding mechanism, an antibody can "tag" the bacteria for attack by other parts of the cow's immune system such as macrophages and neutrophils, or can neutralize its target directly e.g. by blocking a part of the microbe that is essential for either its invasion or survival[10].

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10. *Piepers S. Bradley A. De Vliegher S., Schukken Y., cuestional.*

WHAT DO YOU KNOW ABOUT...

1. Which are the first cells to arrive at the site of an infection?
a. Macrophages
b. Polymorphonuclear neutrophils (PMN)
c. Lymphocytes
d. Cytokines

2. The function of lactoferrin is to:
a. Recognize infected cells.
b. Inhibit iron-dependent bacteria.
c. Stimulate PMNs.
d. act as a chemical messenger.

3. Which of the following statements about Th1 is correct?
a. It is in anti-inflammatory response.
b. It is produced by IgG1.
c. It is very common at the beginning of lactation.
d. All the above are incorrect.

4. J5 vaccination
a. Produces long-term immunity.
b. Reduces the severity of clinical symptoms.
c. Increases IgG1.
d. Reduces new infections.

5. Which of the following statements about *S. aureus* vaccination is not correct?
a. It is not associated with a reduction in bacterial counts.
b. It produces an increase in serum IgG.
c. It produces an increase in in milk IgG.
d. It is based on increasing antibody titres against SAAC. (slime associated antigenic complex).

6. Sofie Piepers' study concludes that
a. Vaccinated animals show higher levels of IgG in blood.
b. Vaccinated animals show higher levels of IgG in milk.
c. Vaccinated animals have fewer inflammatory problems in the udder.
d. All the above are correct.

7. Which of the following actions does NOT support innate immunity?
a. Bad NEB
b. Cow comfort
c. Supplementing with trace minerals and vitamins just before calving and at the beginning of lactation.
d. Teat end scoring

8. What can we do to improve acquired immunity?
a. Improve hygiene management
b. Vaccination
c. Vaccination and dry-off treatment
d. Dry-off treatment

Find the answers below:

1b 2b 3c 4b 5a 6d 7a 8b

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Environmental control, keeping the cows clean, dry and comfortable

INTRODUCTION

Environmental control is the first key point to be evaluated and fixed in every single milk quality program. Despite the information available about it, environmental mastitis is still a major issue all over the world. The goal is to always keep our lactating, dry and transition cows clean, dry and comfortable in order to minimize teat exposure to environmental bacteria and thereby reduce the risk of intramammary infections (IMI).

Bedding is the major source of environmental bacteria, as teats are in direct contact with bedding when cows are lying down, but we should not forget alleys, holding areas, parlours, etc, because manure splashed on to walking cows will help to increase teat exposure and dirty hooves will easily contaminate clean bedding and teats when cows are lying down.

There are different bedding materials worldwide, and the first choice will depend on availability, cost and type of facilities (bedded pack pen or freestalls).

Although there are some materials with a lower environmental bacterial load, bedding maintenance is even more important than the material that has been chosen. For this reason, we must schedule a bedding maintenance program for all the facilities to ensure a good environment.

Other critical points for environmental cleanliness will be the stocking density of the animals, design of the resting area (freestalls or bedded pack pens) and ventilation of the barn.

HOW TO EVALUATE THE ENVIRONMENT?

First we need to know how clean our cows are. Most of the time, we follow our "instinct" when we evaluate the farm environment just by taking a look around at the farm. Although this is a first approach we can carry out a better and more accurate evaluation by looking at the animals in the milking parlour.

By following this chart (figure 1), we can obtain accurate figures about how clean our cows are when they arrive at the milking parlour. Cows with a score of 3 and 4 have a 1.5 times greater probability of acquiring a new infection compared with a score of 1 and 2. There is no clear goal in terms of cleanliness, but <20% having a score of 3-4 would be a good suggestion. Ideally, we should evaluate udder hygiene in 20% of cows in each pen or all the cows in small herds.

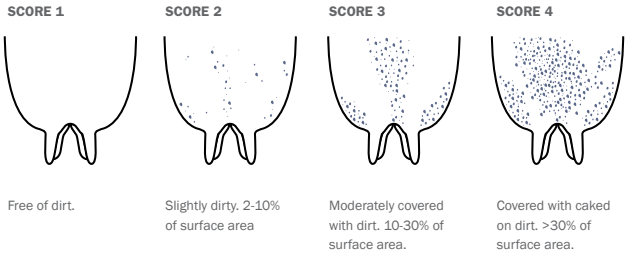

Figure 1. Adapted from Udder Hygiene Scoring Chart. P. L Ruegg 2002.

Some research has also been carried out on the amount of bacteria in the bedding that can lead to major problems of clinical mastitis. Usually we talk about:

- Minimal risk: fewer than 300,000 cfu/g.
- Moderate risk: 300,000 to 1,000,000 cfu/g.
- High risk: more than 1,000,000 cfu/g.

It is interesting to take samples from clean bedding material and then from used bedding material from the rear part of the freestall. Select at least 6 or more stalls from which to take samples and for clean material sample different areas of the bedding pile. Refrigerate samples before sending them to the laboratory. Ideally they should be analyzed within a maximum of 48-72 hours. Freezing samples is not a good option as the freezing and defrosting process can potentially break many bacterial walls, leading to a misinterpretation of the results.

The problem is that research relating to these bacterial counts and clinical mastitis is very old and there is a lack of specific knowledge at the present time. In some cases, for example compost bedding, we can find a very high bacterial count but very few problems with milk quality. Further research is necessary at this point.

BEDDING MATERIALS

We can divide bedding materials or bedding surfaces into two groups. Inorganic or organic.

Inorganic materials can be sand, lime, calcium carbonate etc. and organic materials can be straw, sawdust, compost etc. Obviously, inorganic material allows lower bacterial growth and is preferable for udder health.

Also, in the case of freestalls, these can be divided into those with mattresses and deep bedded freestalls. Deep bedded freestalls provide much better cow comfort than mattresses.

Mattresses: Some farmers like this system because initially it helps to reduce bedding costs and because they think that it will reduce maintenance work. But this is not true. Initial costs are quite high and a mattress system does not mean that neither bedding nor maintenance nor cleanliness are needed. Without bedding, mattresses get wet and moisture is essential for bacterial growth. So using small particle bedding such as sawdust or chopped straw helps to keep it dryer and more comfortable. Also, lime or calcium carbonate are very useful for reducing moisture on the mattress. There is no agreement about the amount of calcium carbonate to be added, but between 250-500 gr/stall daily would be a good recommendation. On the other hand, some types of mattress could compromise cow comfort as some of them are very hard or become rough or slippery with ageing, promoting hook lesions and lameness.

Ideally, we should provide 3-5 cm of bedding material on the mattress to improve cow comfort and avoid wetness.

There are different types of mattress available on the market:

- Solid carpet of rubber. Usually 2-4 cm thick. These are the hardest ones. This type of mattress is very uncomfortable for the cows and will create hoof and joint problems.
- Chopped rubber mattresses. Usually 5-10 cm thick. These provide a better cushion for the cows but with time they also become harder and uncomfortable for the cows.
- Latex or polyethylene foam mattresses. Usually 5-10 cm thick. Good cushion and usually do not become harder with time.
- Water mattresses. This type of mattress is very comfortable for the cows but they have to be really good or a water leak can occur.

Sand: From a bacteriological point of view, sand is the first choice for the reduction of IMI caused by environmental bacteria. As an inorganic material, it does not support the growth of environmental bacteria and it usually has lower bacterial counts than organic materials, so teat exposure is lower, as is the risk of IMI. However, we must consider availability and price. Also, it needs special manure handling systems which tend to be expensive.

When new facilities are planned, arrangements can be made for the use of sand, but to use sand on a farm which is not prepared for this can be a huge problem.

It is also possible to recycle the sand to reduce bedding costs. Some farmers leave used sand in a pile for a few months and organic matter from manure is composted and reduced. Others, in larger units, prefer to wash the sand. In both cases, the goal is to obtain sand with less than 2% of organic matter.
Some types of fine sand become harder when in the freestall because of the weight of the cow. In this case, it is important to till it once a day or every two days. This is also important in order to avoid small piles of sand between freestalls. With this operation we have a better use of the sand and the comfort of the freestall is improved.

Straw: This is the most common material and the only one available in some places. There is scientific evidence that straw is a perfect material for *Streptococcus* species to live in and IMI´s caused by *Streptococcus uberis* are very common on farms using straw as bedding.

Straw gives a very good cushion for the cows but it does not absorb moisture like other materials can.

Once again, maintenance is the key point. For straw bedded freestalls, new straw needs to be put down at least 2 or 3 times per week. In straw bedded pack pens, clean straw has to be put down every day and everything cleaned once a week.

Sawdust and wood shavings: Wood products provide good cow comfort and cleanliness of the cows in both systems (freestalls and bedded pens). Availability and prices in different areas will determine if they are attractive for your farm.

As an organic material, sawdust and wood shavings can be an important source of mastitis causing bacteria. Green sawdust can be an important source of *Klebsiella*, *E.coli* and other coliforms. Sawdust from old furniture or construction can be a useful material. It is much drier and contains fewer bacteria.

The finer this material is chopped, the better absorption capacity it has, but at the same time bacterial growth is increased. At this point, fine sawdust can contain more bacteria than wood shavings but also have greater absorption capacity.

Compost: This system is becoming more and more popular, especially in bedded pens. The system works by applying 30-40 cm of bedding material such as sawdust or wood shavings over the whole resting area. Then, once or twice a day during milking time when the cows are not in the pen, the entire bedding area has to be aerated with a cultivator. Once or twice a week, more sawdust can be added to maintain the structure and reduce moisture in the resting area. Finally, all the bedding can be removed once or twice a year to be spread on the fields.

Environmental control, keeping the cows clean, dry and comfortable

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Some farmers never remove all the bedding, but only part of it, and make this system continuous. For the best performance with this type of bedding we need very good ventilation and the correct density of animals.

Recycled manure solids: Some farmers use dried manure directly from the screw. This manure can be fresh manure or manure from methane digesters for the production of biogas and electricity. Dry Matter content is a critical point and it should be >30% before application. Once again, it is an organic material and bacterial contamination and growth can be very fast.

So it is important to add new material daily and to have a high turnover of bedding material. It is also important to till it over every day when the cows are in the parlour in order to aerate it and reduce moisture.

Apart from the type of bedding material that can be used on the farm, there are also many additives available onthe market for reduction of moisture and the control of bacterial growth. The most commonly used additive is hydrated lime or also calcium carbonate. The goal is to reduce moisture and increase bedding pH. It has to be applied daily, because it has a very short effect, usually less than 24 hours, and usually it is applied on top of the bedding. In the rear third of the freestall or on all bedding surfaces for bedded pens. Research indicates that mixing the lime with the bedding material prior to application is most effective in reducing bacterial growth.

Hydrated lime or calcium carbonate are very cheap and they can be used without restriction. Some other commercial powders with disinfectants for bedding tend to be quite expensive and farmers use them in small quantities, so ultimately they are not very effective in reducing the bacterial count. Daily maintenance is absolutely essential and at each milking time, when cows are in the parlour, freestalls must be cleaned of faeces and wet areas covered with bedding from the front part of the freestall.

As regards alleys, ideally alley scrapers should be run 24 hours/day to eliminate faeces and humidity. Evaluate how clean the alleys are and how many times alley scrapers are running daily. The more, the better.

ENVIRONMENT IN DRY COWS

Dry cow pens are sometimes the forgotten pens, because they do not produce milk. This is a great mistake, since many new clinical cases that occur during the first 100 days in milk originate in the dry period, as reported in scientific studies.

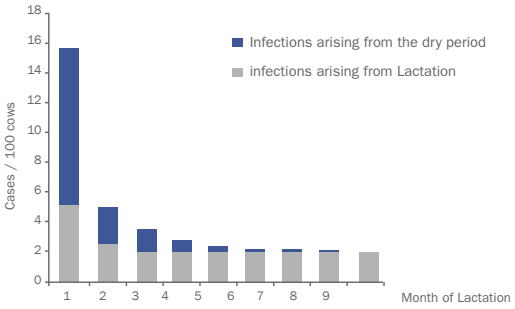

Figure 2. Adapted from A.J. Bradley. Vet Clin Food Anim 20 (2004) 547 568.

So, prevention of infection by environmental bacteria starts with dry cows. From a milk quality standpoint, we tend to say that new lactation does not start when the cow calves. It starts when that cow is dried off. The highest risk of new infection is just after drying off and around calving, as shown in the graph below, so environmental conditions in dry cows and close up cows must be at least as excellent as for the lactating animals.

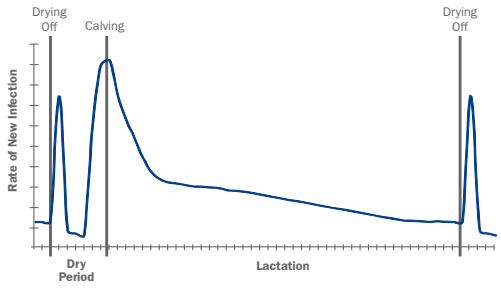

Figure 3. Adapted from A.J. Bradley. Vet Clin Food Anim 20 (2004) 547 568.

FACILITIES DESIGN

Basically there are 3 types of facility where the cows can rest:

- Tie stall
- Free stall
- Bedded pack pens

Regardless of the bedding used, design, stocking density and maintenance play a huge role in environmental control in this kind of facility. Remember that we have to provide a clean, dry and comfortable environment for the cows if we want to make them perform correctly. In terms of milk production, but also for control of diseases such as mastitis or lameness.

A poor freestall design will compromise cow comfort and will make it more difficult to keep bedding clean. If freestalls are not comfortable enough, cows will look for another place to lie

down, such as in the alleys. Below right is a design for freestalls:

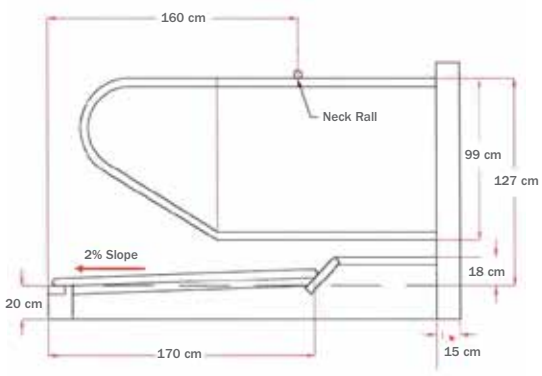

Figure 4. Stall dimensions . Adapted from Andy Johnson.

As regards density, for pens with freestalls, the rule of thumb is one freestall per cow in the pen. Despite this, we can find farms working with 2-5% extra cows with no problems. Obviously this situation is not advisable, but we do find it on some farms. At this point, excellent bedding maintenance is absolutely essential. More than 5% overdensity will lead to environmental problems. For the bedded pack pens, recommended areas per cow are as follows:

- Lactating cows: 10 m2/cow
- Dry off cows: 8 m2/cow
- Close up cows: 11 m2/cow
- Maternity: from 13 to 18 m2/cow

These recommended areas are useless if good maintenance and excellent ventilation are not provided. Also, heat stress will play a major role if the cows are not distributed evenly along the pen.

When cows suffer heat stress they tend to group in one area of the barn. Usually where the main wind comes into the barn, but sometimes they group in a corner just because they are stressed and they react as some ruminants do in the face of a stressing situation, by grouping. The bedding in these areas becomes very wet and dirty and it is an important source of bacteria. We can see the same situation when we have a great many flies in the barn. Flies are extremely annoying for the cows and must be controlled in order to prevent cows grouping.

Water trough location is also very important. It is common in old facilities to find water troughs inside the bedding areas, producing very wet areas surrounding them, especially in summer time. The areas around water points are also an important source of bacteria.

For freestall pens, water points must be installed in the crossing alleys if a barn has 3 rows of freestalls. These crossing alleys should measure at least 4 to 5 metres in width. For a 2-row barn, the water point must be in the rear alley. For bedded pens, water points must be out of the resting area, in the alley. Also the rear part of the water point can be protected with a piece of stainless steel so as not wet the resting area.

Correct ventilation is another important issue. As bacteria need humidity to grow, providing fresh and clean air will reduce the bacterial count of the bedding. Cows will also distribute evenly all along the resting area. Of course, we must first increase natural ventilation, but also be prepared to install fans when necessary.

TAKE-HOME MESSAGES

- Cows must be clean, dry and comfortable at all times.
- Choose the right bedding material. Inorganic materials are always better than organic if we are thinking about milk quality.
- Other factors such as availability, price and manure handling facilities will determine one material or another.
- Very good maintenance can reduce the effects of bad bedding material. Bad management can destroy the benefits of good bedding material. Proper management is the most important aspect of cows' bedding.
- Cow hygiene is not a choice. It has to be a farmer's commitment.

Environmental control, keeping the cows clean, dry and comfortable

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WHAT DO YOU KNOW ABOUT...

1. What is the best bedding material from a bacteriological point of view?
a. Straw
b. Recycled manure solids
c. Matress
d. Sand

2. What is more important, bedding material or bedding maintenance?
a. Material
b. Maintenance

3. How can we evaluate environment?
a. Checking cow cleanliness using the udder hygiene scoring chart. 80% should score 1-2.
b. Checking cow cleanliness using the udder hygiene scoring chart. 80% should score 3-4.
c. Checking cow cleanliness using the udder hygiene scoring chart. 20% should score 1-2.
d. Checking milking parlour cleanliness.

4. What kind of bacteria grows easily on straw bedding farms?
a. *Streptococcus spp*
b. *E. coli*
c. *Staph. aureus*
d. Mycoplasma

5. What kind of bacteria grows easily in sawdust or shavings bedding farms?
a. *Streptococcus spp*
b. *Mycoplasma*
c. *Staph. aureus*
d. Klebsiella and other coliforms

6. What is the recommended bedding area per cow in different stages, lactation-dry-close up and maternity?
a. 6-6-6-6
b. 8-6-10-10
c. 10-8-11-13 to 18 m2
d. 10-8-6-6

7. In Dry Manure Solids bedding... what is the critical Dry Mater content before being applied?
a. <30%
b. >30%
c. >50%
d. It is not important

8. Good bedding material and good maintenance are absolutely essential. But, what other factors play an important role in ensuring a good environment and cow cleanliness?
a. Alley width, ventilation, bedding space, stocking density.
b. Feed bunk space, alley width, ventilation, bedding space, stocking density.
c. Design of freestalls, ventilation, bedding space, stocking density and heat stress.
d. Design of milking parlour, design of freestalls, ventilation, bedding space, stocking density.

9. In straw bedded pack pens, how often should they be cleaned out if we don't till it over?
a. Daily
b. Once per week
c. Once per month
d. Never

10. Is environment important in dry cows?
a. Yes
b. No

Find the answers below:

1d, 2b, 3a, 4a, 5d, 6c, 7b, 8c, 9b, 10a

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Checking the Milking Parlour to increase milk quality

DESCRIPTION OF THE MILKING PARLOUR

Everybody knows that to get milk from a dairy cow, the cow has to be milked. Use of milking machines is the easy way to rapidly and efficiently remove the milk without damage to the teat or gland and with minimal risk of the transmission of pathogenic microorganisms that might cause udder disease.

The basic milking machine functions are:

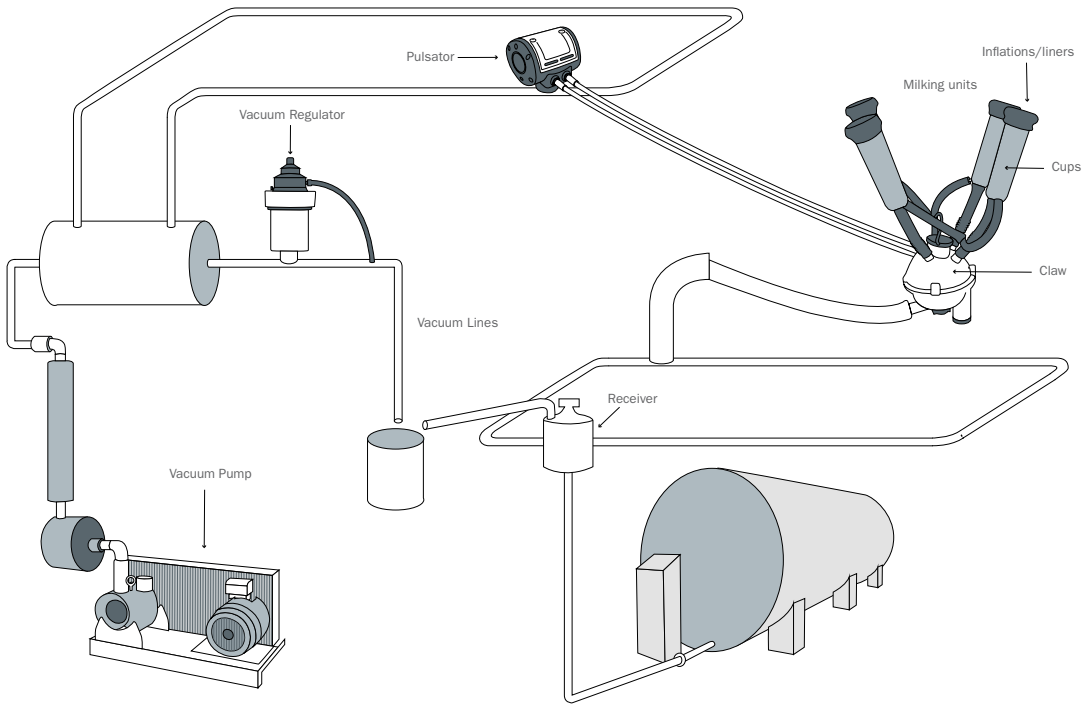
- Removal of milk from the cow by vacuum differential
- The milk then flows to the receiver jar by gravity
- The milk is pumped from the receiver jar into the bulk tank

The milking system consists of the following parts:

- **Vacuum pump:** the pump removes air and creates the vacuum.
- **Vacuum regulator:** The vacuum regulator admits atmospheric air in and out of the system to keep the vacuum at a predetermined set level. If the vacuum gets too high, the vacuum regulator will open and let air into the system to lower the vacuum.
- **Pulsator:** The function of the pulsator is to allow intermittent massage of the teat end to prevent swelling. It does this by alternating between a partial vacuum (milking phase) and atmospheric air pressure (massage phase). Pulsator ratios of 100%, 50/50, 60/40 and 70/30 are typical ratios. The first number refers to the amount of time the pulsator draws vacuum to open the liner and withdraw milk. The second number indicates the amount of time it admits atmospheric air to collapse the liner and massage the teat. Uniform pulsation milks all 4 quarters simultaneously. Alternate pulsation milks two quarters at a time, while the other 2 quarters rest. Dual pulsators allow the quarters to be milked at different ratios, such as 60/40 behind and 50/50 in the front quarters.
- **Receiver:** Milk flows by gravity through the milk line and into the receiver jar. The receiver jar serves as a small holding reservoir until the milk can be pumped into the bulk tank for cooling and storage. The valve between the receiving jar and the milk pump should not admit air. If a bubbling action occurs in the receiver jar, air is leaking past the valve and it should be corrected or replaced.
- **Milking unit:** The machine includes teat cups that are in contact with the cow's teats and remove the milk, a claw where milk pools as it is removed from the four teats, vacuum tubes that provide vacuum to the teat cups and a milk tube that removes milk away from the claw, a source of vacuum for the machine. Connecting tubes are divided into short milk tube (liner to claw), long milk tube (claw to milkline), short pulse tube (shell to air fork) and long pulse tube (air fork to pulsator).

Figure 1. The six basic parts of a milking system.

- The Vacuum Pump
- Vacuum Regulator or Controller
- Receiver
- Pulsator
- Milking units (claw, cups and inflations)
- Vacuum Lines



EVALUATION OF THE MILKING PARLOUR

An initial simple check of the milking machine can be by visual inspection, looking at the most important parts of the milking parlour.

The vacuum regulator requires regular checking, cleaning and maintenance to maintain its designed level of performance. The most common pulsation faults include cracks or splits in the pulse tubes, foreign material (dirt, grit, straw, feed particles or insects) under the pulsator valve seats or lodged in the air inlet ports.

Liners should have no cracks in the short milk tube connecting to the claw, and no surface crazing or swelling evident on the mouthpiece lip or inner barrel. Milk tubes and droppers, short milk tubes and short pulse tubes should be inspected for wear, cracks, tears, or changes in cross-sectional area due to kinking, distortion or swelling due to fat absorption.

Visually inspect at least 20% of the liners and short milk tubes for cracks or splits (NMC, 2008).

After these easy inspections, milking machine equipment can be evaluated. Why is it so important to understand if the milking machine is working well? At the 1987 International Mastitis Symposium in Montreal, Canada, the question was asked “What percentage of all infections are due to milking machine factors?” The answers given were “we don’t really know”; “probably quite low”; and “anywhere between 0% and 100%” (NMC). Can we provide any more definitive answers today? Informed estimates of direct and indirect milking machine effects range from about 6% to 20% of the overall new mastitis infection rate. The direct effects (including bacterial transport, cross-contamination and impacts) might account for about 10% of new infections on most farms. Indirect effects (including effects on the health of the teat canal, teat tissues and skin) might account for another 10% in an average herd. However, it is difficult to go beyond these broad-brush estimates (Mein at al., 2004).

The use of testing equipment and procedures for measurement of vacuum levels and vacuum changes during milking has been defined loosely as “dynamic testing”. In 1996, Reinemann et al., proposed a new way to classify tests.

- Dry tests: tests conducted with the machine running but not milking and only air flowing through the machine.
- Wet tests: tests performed with the machine running but not milking with both air and liquid (water, milk or artificial milk) flowing through the machine using flow simulator or artificial udder.
- Milking time tests: observations or measurements made while milking live animals.

As new test methods are developed, new terminology is required to describe these various types of tests. The term “static testing” has traditionally been used to describe tests that are performed with the machine running but only air flowing through the system. This term is misleading and should be dropped. During these types of tests, air is moving into and through the system, liners are opening and closing. The key feature of so-called “static testing” is that the milking machine is running but not milking. More particularly, no liquid is flowing through the system. The system is dry. Most dry tests measure vacuum levels, air flow rates, and the cyclic vacuum changes in the pulsation system (Reinemann, 1996).

STATIC OR DRY TEST:

The term “static testing” has traditionally been used to describe tests that are performed with the machine running but only air flowing through the system (Reinemann, 1996).

The static test includes the following:

- Vacuum levels in the plant: Vacuum levels are checked at various locations throughout the plant to ensure that there is no significant loss of vacuum between the pump and the teat end, and that the plant is set at the correct level. A drop in vacuum level would indicate that air is leaking into the system. The accuracy of the vacuum gauge is also checked
- Vacuum reserve: Adequate vacuum reserve is needed to ensure that stability of pressure is maintained in the plant throughout milking. The ISO has made recommendations for vacuum reserve. It must be remembered that these are minimum recommendations, and ideally new plants should exceed these levels significantly. Systems with a low vacuum reserve will have difficulty in maintaining stable vacuum levels during milking. This may result in an increased number of liner slips and irregular vacuum fluctuations, which may affect the incidence of mastitis and poor milkout. The vacuum pump should have sufficient reserve capacity (known as the Effective Reserve or the Manual Reserve) to cope with accidental air admission through the teatcups during milking. The adequacy of reserve pump capacity can be estimated in the following way. Note the vacuum level (preferably, in or near the receiver) with all units shut off. Then, open the vacuum shut-off valves to one unit (or two units in systems with more than 32 units). If the vacuum level does not fall more than 2 kPa, then the Effective Reserve is likely to be

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adequate. If this test is made under the same conditions each month, and the vacuum level recorded for 1 or 2 clusters is fully open, then gradual changes in reserve pump capacity due to air leaks, pump wear or regulator malfunction can be monitored systematically (NMC, 1998).

- Regulator function: It is important that the regulator functions correctly so that a stable vacuum level can be maintained throughout milking. Regulators commonly become blocked with dirt, thereby reducing the amount of air leaking into the system, but occasionally the mechanism become defective.
- Pulsation: The most common pulsation faults include cracks or splits in the pulse tubes, foreign material (dirt, grit, straw, feed particles or insects) under the pulsator valve seats or lodged in the air inlet ports (NMC, 2006).
- General condition of the plant, rubberware etc: The plant should be examined for any perished rubberware, leaky valves, etc., and its overall condition noted. Liner condition should be assessed and the frequency of change checked to ensure that the liners are replaced at the correct intervals.

DYNAMIC OR WET TEST:

Proper pulsator function is critical to the success of the milking process. Milking-time tests are the most direct method to determine the adequacy of vacuum production and vacuum regulation in any milking system. More detailed testing can be used to diagnose the cause of failure for either of these tests.

Pulsation Rate

The number of times per minute that the pulsator alternates between the milking and massage phase is called the PULSATION RATE. Rates vary from about 40 to 80 pulsations per minute, depending upon the manufacturer. A rate between 50 to 60 is usually recommended.

Pulsation Ratio

The ratio of time the inflation is in the milking phase compared to the time it is in the massage (rest) phase is called the PULSATION RATIO (or milk to rest ratio). Ratios vary by manufacturer, from 50:50 to about 70:30. Cows will usually milk slightly faster with a wider ratio, such as 70:30. However, the longer milk phase and shorter rest phase may cause teat end trauma and damage if the milking equipment is not working properly and if good milking practices are not followed. Ratios near 60:40 are less likely to contribute to problem situations.

Test equipment: A single-channel unit or multi-channel vacuum recorder and at least 4 teatcup plugs are required for correct testing of pulsators. Because most pulsator testers include an option for measuring vacuum level, the pulsator test unit can be used as an accurate digital vacuum gauge and for measuring vacuum stability in the milkline and claw.

Pulsator Testing: These tests are done with milking units connected, pulsators operating and liners fitted with teat cup plugs. The objective of these tests is to determine if the pulsation system and all pulsators are operating according to the manufacturer's specifications. Pulsation testers are used to determine the pulsation rate and the duration of the four phases of pulsation. The main parameters of interest for pulsators are:

- Pulsation: The ISO standard definition of the pulsation ratio is the proportion of the opening and open phases (a+b) to the closing and closed phases (c+d) of the pulsation cycle. From about 1967, pulsation was defined more specifically as 'cyclic opening and closing of a teatcup liner'. At about the same time, the term 'pulsation ratio' was used in a few scientific papers to define the proportion of time - within each pulsation cycle - that the liner was more than half open. In the USA, this concept was adapted to define the 'milk:rest ratio' for a given liner based on its 'touch point'. A milk:rest ratio of 60:40, for example, implies a 'milking phase' of 60% of each pulsation cycle followed by a 'massage phase' of 40%.

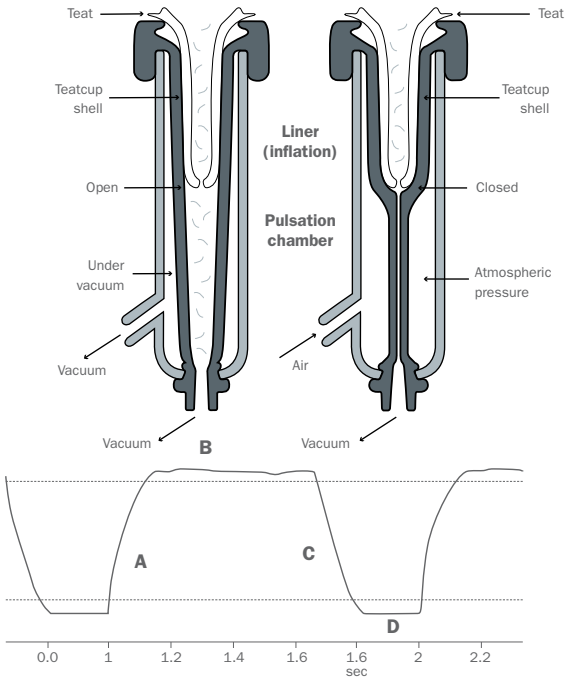

Figure 2. Phases of pulsation.

The two phases are assumed to start and stop at the point where the opposing liner walls first touch each other when measured with no teat in the liner. Methods for defining pulsation in terms of liner wall movement rather than pressure changes have also been proposed (Spencer and Jones 2000; Spencer 2003; Mein and Reinemann, 2009).

- The Pulsator Ratio should not differ by more than 5 percentage units from the manufacturer's specifications or from one pulsator to another.
    - The B Phase (milking or liner-open phase) of pulsation should be at least 30% of the cycle.
    - The D Phase (massage, rest or liner-closed phase) of the pulsation cycle should not be less than 15% and not less than 150 milliseconds (Reinemann et al., 2001).

    For common liner types, this balance is achieved with a D-phase of 150 to 200 milliseconds and a B-phase of 500 to 600 milliseconds. The A and C phases of pulsation can vary according to the volume of the pulsation chamber, length of long pulse tubes and restrictiveness of pulsator air ports (Reinemann, 2010).

Vacuum level

ASAE specifies and ISO recommends that the performance criteria for vacuum stability in a milking machine is that the vacuum drop in or near the receiver does not exceed 2 kPa (0.6"Hg) during normal milking. Normal milking is considered to be the time that milking units are attached to cows including events such as teatcup attachment and removal, liner slips and cluster falloff. In addition, the vacuum should not drop more than 2 kPa (0.6"Hg) below the receiver vacuum at any point in the milkline for at least 95% of the normal milking period. The most useful sites of measurement are in the milkline, in or near the receiver (if necessary), and in the claw. Vacuum at these sites should be recorded while the system is under full milk and air flow conditions, that is, while clusters are being attached, while all clusters are on cows, and then as clusters are detached.

Recommendations for test equipment have been presented previously for milking time tests as specified by the new ASAE standard S518 and Practice ASAE EP445, "Test equipment and its application for measuring milking machine operating characteristics," and the revised draft international standard DIS/ ISO 5707. These tests are performed in the receiver, milkline, and claw. The National Mastitis Council recommends measuring the bandwidth (maximum - minimum) claw vacuum during milking in the new "Procedures for Evaluating Vacuum Levels and Air Flow in Milking Systems" (Reinemann et al., 1996, Reinemann, 2007). Previous studies provided recommendations for testing vacuum stability in the receiver, milkline and the range of vacuum at the claw outlet during milking (Muthukumarappan et al., 1995, Reinemann et al., 1996). The range of vacuum fluctuations (maximum-minimum) prescribed in the NMC, ASAE and ISO standards and recommended accuracy for the milking-time measurements are as follows:

Test	Ranger (kPa)	Accuracy (kPa)
Receiver Vacuum	< 2 kPa	+/- 0.2 kPa
Milkline Vacuum	< 2 kPa	+/- 0.2 kPa
Claw Vacuum	7 to 10 kPa	+/- 1.0 kPa

Table 1. Range of vacuum fluctuations.

Operating vacuum is not a sufficient measure to characterize the quality of milking. Teat-end vacuum, milk flow patterns and the response of teats to the milking process are preferred indicators for evaluating milking system design and settings (Haeussermann and Hartung, 2010). Teat-end vacuum is influenced by milk flow level, milking system, system components and milking settings. Thus, the optimal teat-end vacuum curve should show a vacuum between 26 to 39 kPa in the maximum vacuum phase (b) and a much lower and steady teat-end vacuum in the minimum vacuum phase (d). During the complete time-span of the b-phase, the vacuum should be as constant as possible. High teat-end vacuum (33 to 39 kPa) should only be adjusted when the milk flow is higher than 0.8 L/min per quarter, because only then is the high vacuum also required for the transportation of the milk, resulting in more efficient milking (Ströbel et al., 2013).

TEAT STATUS, EVALUATION:

The teat sphincter and teat canal are important primary barriers against pathogen invasion into the udder. Thus, it is essential that such structures should be in perfect physical and hygienic conditions to prevent intramammary infection (IMI) (M. de Pinho Manzi et al., 2011).

The "Teat Club International" suggested a range of teat condition scores for the evaluation of field data and divided them into short- (single milkings), medium- (few days or weeks), and long-term effects (several weeks) (NMC,2004).

Short-Term Changes In Teat Condition

- Faults in milking machines or milking management are the primary cause of short-term changes in colour (pink, red or blue coloured), firmness, thickness or swelling of teats (no ring, garter mark or palpable ring), or degree of

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"openness" of the teat orifice (closed or open more than 2mm) (Mein et al., 2001).

Medium-Term Changes In Teat Condition

Machine-induced changes in the incidence of petechial haemorrhages or larger haemorrhaging may occur immediately or may take several days before becoming evident. Changes in teat skin condition associated with harsh weather or chemical irritation may take a few days or weeks. When teats were intentionally irritated with a harsh chemical, the irritant effect was maximized after 1-3 days. Progressive healing from severe teat skin and teat-end damage can take 3-5 weeks. More typical degrees of irritation resolve in 10-14 days (Ohnstad et al., 2007).

Long-Term Changes In Teat Condition

The amount of hyperkeratosis varies dynamically, increasing from calving to peak lactation and then decreasing towards the end of lactation (Ohnstad et al., 2007) It also increases progressively with parity (Shearn and Hillerton, 1995; Neijenhuis et al., 2002). Teat end hyperkeratosis is often influenced by seasonal weather conditions. The extent of hyperkeratosis and the degree to which it can be improved is related to teat shape being worse with long, slender or pointed teats. There may, therefore, be a genetic influence (Ohnstad et al., 2007).

To simplify and streamline the procedure, teat condition should be evaluated immediately after the cluster is removed and before application of a teat disinfectant. Mein et al., in 2001, looking at a scoring method developed by Neijenhuis et al. (1998), produced a simplified system for routine field evaluation giving a score to the teat end:

- No ring (N) a typical status for many teats soon after the start of lactation. This category includes teat-ends scored as 1.
- Smooth or Slightly rough ring (S) a raised ring with no roughness or only mild roughness and no keratin fronds. This category includes teats classified as 2.
- Rough (R) a raised roughened ring with isolated fronds of old keratin extending 1-3 mm from the orifice. This category indicates some breakdown in epithelial integrity. It includes teat-ends classed as 3.
- Very Rough (VR) a raised ring with rough fronds of old keratin extending >4 mm from the orifice. The rim of the ring is rough and cracked giving the teat-end a "flowered" appearance. This category includes teat-ends classified as 4 (Mein et al., 2001).

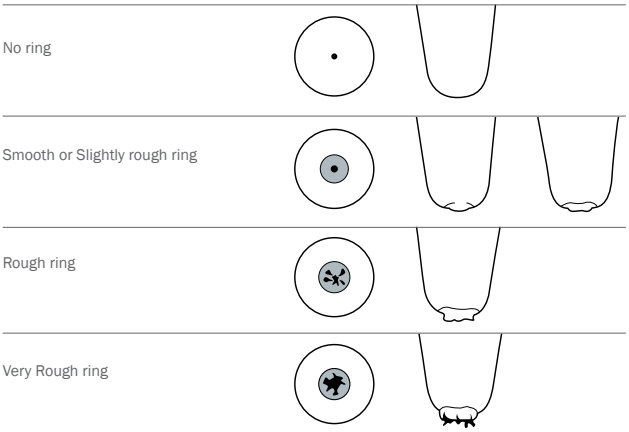

Figure 3: Teat end condition evaluation. Adapted from Mein et al., 2001.

Teat ends with very rough rings with keratin extending more than 4 mm from the orifice (score 4) had the highest chance of developing IMI when compared to the other three categories. There is a clear relationship between intramammary infections and teat end status. (M. de Pinho Manzi et al., 2012).

Score teat condition on all teats of all cows in the herd if time and herd size allow. If time is limited and/or for large herds, score all teats of all cows in herds of up to 80 cows, or a random sample of at least 80 cows in herds with 80 - 400 cows, or at least 20% of cows in herds larger than 400 cows (Mein et al., 2001).

Table 2 summarizes all the relative criteria for evaluation of the results of teat scoring. The use of Table 2 can help with the evaluation and detection of a teat problem in a herd.

Test Condition Measure	Criteria
1. Color	>20% visibly reddened or blu
2. Swelling al Test Base	>20% swelling or palpable ring
3. Swelling al Test End	>20% firm, hard or swollen
4. Openness os Teat Orifice	>20% open
5. Vascular Damage	>10% petechiations
6. Teat End Roughness	>20% Rough & Very Rough (3 & 4)
7. Open lesions	>5% open lesions or cracked skin

Table 2: Teat condition measure and relative criteria. Adapted from Reinemann, et al., Proc. International Mastitis Meeting Nov 2001

WHAT DO YOU KNOW ABOUT...

1. The six basic parts of a milking system:
a. Vacuum pump, Vacuum regulator, Pulsator, Receiver, Milking unit and Vacuum lines.
b. Vacuum pump, Vacuum regulator, Pulsator, Receiver, Milking unit and Milkers.
c. Vacuum pump, Vacuum pulsator, Pulsator, Receiver, Milking unit and Vacuum lines.
d. Vacuum pump, Inverter, Vacuum regulator, Pulsator, Receiver, and Milking unit.

2. Evaluation of the milking parlor, First simple check:
a. Visual inspection of: at least 80% of the liners and short milk tubes for cracks or splits.
b. Visual inspection of: vacuum regulator, pulse tubes, Liners, Milk tubes, droppers, short milk tubes and short pulse tubes.
c. Turn on milking machine and look if something goes wrong.
d. Visual inspection of: vacuum regulator, pulse tubes, Milk tubes, droppers, short milk tubes and short pulse tubes.

3. Measurement of vacuum levels and vacuum changes, Reinemann et al., in the 1996 proposed a new way to classify tests:
a. Static tests, Dry tests, Milking time tests
b. Dry tests, Wet tests
c. Dry tests, Wet tests, Milking time tests
d. Dry tests, Static test, Wet tests, Milking time tests

4. The ISO standard definition of the pulsation ratio is the proportion of the opening and open phases ("milking") to the closing and closed phases ("massage") of the pulsation cycle. Respectively, which phases are milking and massage?
a. (a+b) and (c+d)
b. (a+c) and (b+d)
c. (a+d) and (b+c)
d. None of the three answers reported

5. Respectively B and D phases should be:
a. 30% of the cycle, 500 to 600 milliseconds and 15% of the cycle, 150 to 200 milliseconds.
b. 50% and 50% of the cycle.
c. 15% of the cycle, 500 to 600 milliseconds and 15% of the cycle, 150 to 200 milliseconds.
d. At least 30% of the cycle, 500 to 600 milliseconds and maximum 15% of the cycle, 150 to 200 milliseconds.

6. ASAE specifies and ISO recommends that the performance criteria for a vacuum stability in a milking machine is that the vacuum drop in or near the receiver does not exceed 2 kPa (0.6"Hg) during normal milking. Where do you need to measure it?
a. In the claw.
b. From receiver vacuum at any point in the milkline, in or near the receiver.
c. Only in the vacuum lines.
d. In the claw and closed to the claw.

7. ASAE specifies and ISO recommends that the performance criteria for a vacuum stability in a milking machine is that the vacuum drop in or near the receiver does not exceed 2 kPa (0.6"Hg) during normal milking. When do you need to measure it?
a. While milkers are attaching every single unit.
b. While cows are leaving milking parlor.
c. While clusters are being attached, while all clusters are on cows, and then as clusters are detached.
d. While clusters are detached.

8. The optimal teat-end vacuum curve should show a vacuum between 26 to 39 kPa. In which phases is necessary to have the much lower and steady teat-end vacuum and a maximum vacuum?
a. a and c
b. b and d
c. d and b
d. Need to be constant for all 4 phases

9. The "Teat Club International" suggested a range of teat condition scores for the evaluation of field data and divided them into short, medium and long-term effects. In which one of this categories can be assigned hyperkeratosis?
a. short and medium effect
b. short effect
c. medium effect
d. long effect

10. Looking on a teat end scoring method, evaluated immediately after the cluster is removed and before application of a teat disinfectant, sign the maximum percentage for scores 3 and 4 collected.
a. 20% and 5%
b. 50% and 5%
c. 20% and 10%
d. 20% and 0%

Find the answers below:

1a, 2b, 3c, 4d, 5a, 6b, 7c, 8c, 9d, 10a

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Efficient milking routine for high quality milk

INTRODUCTION

The production of high quality milk depends on the hygiene conditions of cows during the milking process, the udder health programme, and on the efficient cooling of raw milk. During the milking process, some of the critical factors influencing the risk of new intramammary infections are: cleanliness of udders and teats, cow handling procedures and the functioning of milking equipment. Therefore, the main objectives of an efficient milking routine are to produce high quality milk, to reduce the risk of new intramammary infections and total milking time, to minimize stress to cows and to milkers, as well as to improve dairy farm profitability.

MANAGEMENT OF THE MILKING PARLOUR

Efficient management of the milking parlour depends on the specific milking routine and on the parlour configuration. After deciding which routine should be used, milkers should be trained and constantly assessed on the degree of implementation of milking procedures. Milking routine refers to how milkers move through a milking parlour, whilst milking procedures refer to each specific step during milking that is performed by milkers. Traditionally, milking routine can be classified as territorial or sequential. A territorial milking routine occurs when one milker is responsible for all procedures on a set of cows for a specific territory. On the other hand, a sequential milking routine is based on a sequence of milking procedures performed on the same cow by different milkers (for example, one milker performs forestripping and predipping and another milker wipes teats and attaches milking units). A complete milking routine includes procedures such as: forestripping, predipping, drying of teats, timely attachment of milking units and the use of effective post-dipping teat disinfection.

PRE-MILKING ROUTINE

The main objectives of a consistent pre-milking routine are to reduce the bacterial contamination of teats, to detect abnormal milk (clinical mastitis) and to help to stimulate the milk let-down reflex. Therefore, in order to achieve a rapid and efficient milking process, pre-milking procedures should ensure that milking units are applied to clean, dry and stimulated teats.

The milking routine starts with the movement of cows to the holding pen or to the milking parlour. Cows should be managed gently and calmly, in order to reduce stress and to avoid inhibition of the milk let-down reflex. When cows are stressed before milking, there is increased occurrence of defecation and liner slips. Improvement of cow movement to the milking centre may have a beneficial impact on parlour efficiency and can reduce the stress to cows and operators.

One recommended practice to reduce the transfer of a contagious mastitis pathogen between cows during milking is to milk infected cows last (or alternatively to designate a separate milking unit for infected cows) to reduce the transfer of mastitis pathogens during milking. Additionally, it is recommended that milkers wear gloves, because contagious mastitis pathogens, such as *Staphylococcus aureus,* may be present on the milker's hand and may be transmitted to cows during milking. Therefore, the objectives of wearing gloves are to reduce the spread of contagious pathogens, and to protect the milker's skin from contact with chemicals and dirt.

Pre-milking procedures may be performed in three basic categories: none, minimal and complete. Dairy herds using complete pre-milking procedures may have improved milk yield per cow when compared to herds that use minimal procedures. One possible explanation is that when milking units are attached to cows with minimal preparation procedures, the milk flow is reduced for 60 to 90 seconds after the start of milking. Therefore, the decision as to which specific pre-milking procedures should be used depends on the milk quality, milking performance and udder health goals of the farm. When the farm decides to used a complete milking routine, the most frequently used pre-milking procedures used in modern dairy herds are: 1) forestripping, 2) predipping, 3) adequate drying of teats and 4) attachment of units.

a. Forestripping

Even though there is no global consensus on this, the practice of forestripping is recommended for the detection of abnormal milk and other symptoms of clinical mastitis. This procedure is done by manually removing a few strips of milk from each teat to detect signs of abnormal milk. Early identification of clinical cases of mastitis helps to initiate adequate treatment protocols and also to divert this abnormal milk from the bulk tank, which is important for maintaining the bulk tank somatic cell count.

Additionally, forestripping is also beneficial for the stimulation of teats during the pre-milking routine. It is estimated that manual stimulation of teats for 10 to 20 seconds is sufficient to induce a good milk ejection reflex for specialized dairy cows. The mammary gland of dairy cows is composed of secretory

tissue based on alveolar structures containing epithelial and myoepithelial cells, and cisternal cavities and ducts. Before milking, the total milk volume within the mammary gland is distributed approximately 20 to 30% in the cisternal compartment and 70 to 80% in the alveolar compartment. During milking, cisternal milk is readily available for removal, but the alveolar milk is removed only after the contraction of myoepithelial cells in response to oxytocin stimulation. After the start of teat stimulation, oxytocin is released from the posterior pituitary into the blood circulation and reaches its maximum concentration 60 to 120 seconds after the beginning of tactile stimulation of teats. Therefore, in order to have an effective and full milk ejection reflex, teatcups should be applied to cows 60 to 90 seconds after the start of manual stimulation of teats. Good stimulation of teats before milking may improve milking performance, by increasing milk flow rates and reducing milking time, when compared to cows that have not been stimulated.

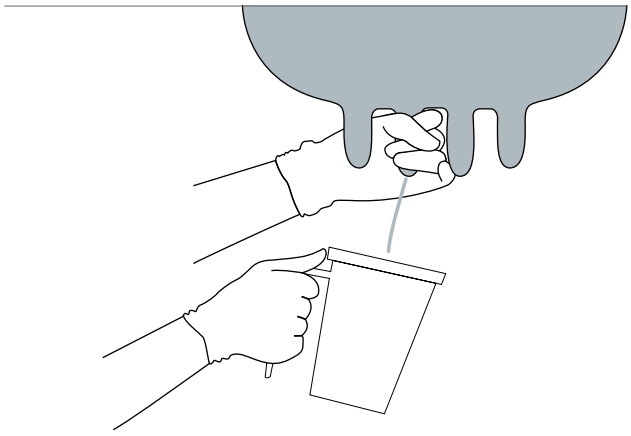

Figure 1. Forestripping.

b. Predipping

There is a direct relationship between hygiene procedures during milking, and bacterial counts of raw milk, and the incidence of clinical and subclinical mastitis. A high bacterial count on teat ends is a major risk factor for new intramammary infections. When cows' teats are exposed to a very dirty housing area there is an increased risk of new cases of mastitis of environmental origin. Therefore, disinfection of teats before milking helps to remove manure and mud and to reduce contamination of opportunistic pathogens that can cause mastitis and increase the total bacterial count of raw milk. The use of predipping of teats using an approved disinfectant is one of the most effective ways to reduce the contamination of teats. In order to achieve effective disinfection, the disinfectant solution should be allowed to remain in contact with teats for approximately 30 seconds, before adequate drying of teats.

Forestripping and predipping are two milking procedures with a positive impact on milk quality, udder health and milking performance. Both procedures stimulate the milk let-down. The order in which forestripping and predipping are performed seems to make no difference in relation to the expected results. However, it is preferable to forestrip first and then to predip teats with a disinfectant solution, in order to reduce the chance of recontamination of teats by milkers' hand. Washing teats should be done only when teats are visibly dirty, using a low-pressure clean water hose.

c. Drying of teats

Complete drying of teats after 30 seconds of predipping is a very effective procedure to reduce bacterial contamination and to avoid residues of disinfectant in milk. The objective of this procedure is to remove dirt, manure, debris and predip residues from the teat skin. The most critical point during the drying process is to clean and dry the teat ends. Wiping off of teats may be done using disposable paper towels or cloth towels that are cleaned and disinfected between each use. Even though cloth towels are more absorbent than paper towels, it should be emphasized that cloth towels should be washed using hot water and sanitizer, and also that they need to be dried at high temperature, in order to reduce bacterial contamination. When teats are not properly dried, wet teats may increase the risk of new intramammary infection and also increase the occurrence of liner slips.

d. Attachment of milking units

The time spent by milkers cleaning and drying the teat surface before milking is called prep time. The critical time for prep time is around 10 to 20 seconds. The prep lag time starts with teat preparation procedures and ends with the attachment of milking units. It is recommended that prelag time should be 60 to 90 seconds, because oxytocin release into the blood circulation reaches peak concentration after 60 to 90 seconds of teat stimulation. Milking routines that result in a prep lag time of more than 3 minutes have negative consequences for milk yield/cow and reduce the milk flow rate. Therefore, a consistent milking routine should focus on a timely attachment of milking units (60 to 90 seconds) to well stimulated teats, aiming to maximize the milk flow rate and to reduce milking time.

When units are attached to the cows, one critical area to monitor in the performance of the milking routine is the unit alignment, because this influences milking performance and efficiency. Teat cups should be attached so as to reduce excessive air admission into the system. Milking units should be aligned so that the outlet of the claw is pointing towards the cow's head (conventional systems) or between the legs of the cows in

Efficient milking routine for high quality milk

Marcos Veiga dos Santos

parallel systems. When units are not properly aligned, cows may have reduced milk flow from individual quarters, which results in an increased risk of teat damage, and liner slips. This situation also results in cow discomfort and increased kicking of the milking unit, liner slips and contamination of the milking unit. Additionally, poor unit alignment increases the time spent by milkers in reattaching units after liner slips.

REMOVAL OF THE MILKING UNIT

The milking unit can be removed manually or by automatic take-offs. With both methods, caution should be exercised so as to avoid overmilking, which results in an increased risk of teat-end hyperkeratosis. Machine stripping or holding down the milking cluster at the end of milking is not recommended, because it increases the risk of teat end damage. To manually remove the milking units, the milker should shut off the vacuum before detaching the unit. Milking units should be removed when the cow has <100 mL of milk per quarter. Strip yields could be performed after detachment of the unit, for 15 seconds, for measurement of the total volume from all four teats in order to evaluate occurrence of incomplete milking (> 20% have quarters with strip yields of > 100 mL of milk).

POST-MILKING ROUTINE

The use of an effective post-milking teat disinfectant is one of the most widely recommended milking procedures to reduce the transmission of contagious mastitis in dairy cows. The objective of post-milking teat dipping is to reduce the contamination of teat skin by mastitis pathogens and to prevent the colonization of the teat canal by mastitis-causing microorganisms. Post-milking teat disinfection should be done as soon as the units are detached. A good post-dipping procedure results in coverage of all teat surfaces that come into contact with liners.

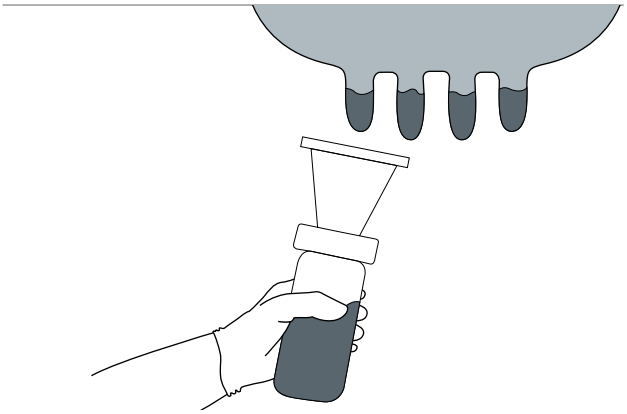

Figure 2. Post-milking teat dipping.

Post-milking disinfectant may be applied by dipping the teats using a clean teatcup. Alternatively, some dairies use teat spraying rather than dipping. When using teat spraying, it should be ensured that there is full coverage of the teat surface by the disinfectant, which sometimes does not happen with teat spraying.

MILKING ROUTINE TIMING AND PERFORMANCE

Milking performance depends on three key elements that interact at milking time: cows, milking facilities, and milkers. Providing comfort, adequate working conditions, and training of the milking staff to successfully perform milking procedures and a consistent milking routine is crucial for the production of high quality milk. Dairy farms where there is frequent training of milkers and a complete milking routine have a higher milking performance and reduced incidence of clinical mastitis. Even though training is very important to milking parlour management, it is not always used on dairy farms. A written protocol (standard operating procedure) for the milking routine helps to inform everyone involved in the management of the milking parlour as to what is expected to be done.

Monitoring the milking parlour management should focus on the performance of milkers, milk quality and udder health in order to identify deficiencies and to correct them quickly. The main key performance indicators for monitoring the milking performance are: a) cows milked per hour; b) cows per person per hour and c) milk harvested per milker. Performance indicators for milking routine are influenced by: cow entry time, pre-milking routine, milking time, milking unit detachment, post-milking routine, and cow exit time. On the other hand, to evaluate the performance of milking routine on the milk quality, the following should be measured: somatic cell count, total bacterial count, coliform count and incidence of clinical mastitis.

ADDITIONAL READING

Fuhrmann, T. 2002. Quality milk starts with quality management. Proc. Annual Meeting-National Mastitis Council National Mastitis Council. 41: 131-139.

Rasmussen, M.D. 2000. A Review of Milking Preparation: The Science. National Mastitis Council Annual Meeting Proceedings. 39: 104-110.

Ruegg, P. L. 2004. Pre-milking cow preparation-Secret methods of producing high quality milk. In: Proc. of the Regional Meeting of the National Mastitis Council. 44: 34-40.

WHAT DO YOU KNOW ABOUT...

1. With regard to milking routine and milking procedures, choose the correct answer:
a. Milking routine refers to specific steps taken during milking.
b. Milking procedures are related to the movement of milkers through a milking parlour.
c. Milking procedures commonly used are sequential and territorial milking.
d. Complete milking procedures include: forestripping, predipping, drying of teats, timely attachment of milking units and post-dipping teat disinfection.
e. Territorial milking is based on a sequence of milking procedures performed on the same cow by different milkers.

2. The main objectives of a consistent pre-milking routine are to:
a. Detect subclinical mastitis and improve milk quality.
b. Prevent clinical mastitis by reducing teat end contamination.
c. Improve milk yield and maintain teat end health condition.
d. Reduce contagious mastitis.
e. Reduce the contamination of teats, detect clinical mastitis and stimulate milk let-down.

3. Foremilking is used with the objective of detecting what?
a. Teat end lesions
b. Clinical mastitis
c. Milking machine failure
d. Subclinical mastitis
e. Teat end contamination

4. A complete milking routine includes the following sequence of recommended pre-milking procedures:
a. 1) forestripping, 2) adequate drying of teats, 3), predipping and 4) attachment of units.
b. 1) washing of teats, 2) predipping, 3) adequate drying of teats and 4) attachment of units.
c. 1) predipping 2) forestripping, 3) washing of teats and 4) attachment of units.
d. 1) washing of teats, 2) predipping, 3) forestripping and 4) attachment of units.
e. 1) forestripping, 2) predipping, 3) adequate drying of teats and 4) attachment of units.

5. Disinfectant solution should be allowed to remain in contact with teats for approximately ____and pre lag time should be____:
a. 10 to 20 seconds and 60 to 90 seconds.
b. 30 seconds and 60 to 90 seconds.
c. 60 to 90 seconds and 30 seconds.
d. 1 minute and 2 minutes.
e. 2 minutes and 1 minute.

6. With regard to attachment of milking units, choose the correct answer:
a. Prep time should be around 30 to 90 seconds.
b. Pre-lag time should be 60 to 90 seconds.
c. Oxytocin release into the blood circulation reaches peak concentration after 10 to 20 seconds of teat stimulation.
d. Teatcups should be attached quickly to avoid cow discomfort.
e. Poor unit alignment reduces cow discomfort and kicking off the milking unit.

7. To avoid overmilking, the milking unit should be removed:
a. When a cow has <10 mL of milk per quarter.
b. By machine stripping or holding down the milking cluster at the end of milking, for 15 seconds.
c. After milk flow has ended to prevent hyperkeratosis.
d. Strip milking should be done in all cows, after unit detachment.
e. None of the above.

8. Which of these milking procedures reduces transmission of contagious bacteria from an infected cow during milking?
a. Forestripping
b. Pre-dipping
c. Cluster washing
d. Post-milking disinfection
e. Washing hands before milking

9. Post-milking teat disinfection should be done:
a. To achieve at least 50% coverage of teat surfaces.
b. To cover all of the teat surfaces.
c. To reduce the transmission of environmental mastitis.
d. Only in herds with contagious mastitis problems.
e. Only in herds with environmental mastitis problems.

Find the answers below:

1d, 2e, 3b, 4e, 5b, 6b, 7c, 8d, 9b

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Management of Somatic Cell Count (SCC)

INTRODUCTION

a. What is mastitis?

Mastitis is an inflammation of the udder which can be defined as either clinical or sub-clinical. What is the difference? For the cow it is just the degree of inflammation. Is it sufficient for the changes in milk to be seen with the naked eye (clinical) or is a (laboratory) test required to detect it? How good is the milkers eyesight?

Simple Mastitis Control

Prevent new infections	**BACTERIOLOGY**
Management	What bugs are involved?
Milking cow	We know where they live
Environment/housing/hygiene	Change management to reduce
Milking routine	new infections with these bugs
Dry cow	
Environment/housing/hygiene	
Internal teatseal	**RECORDS – PATTERN RECOGNITION**
Vaccination	Where do they originate
	Dry period
Remove existing infections	Lactation
Treatment	Environment
Lactating cow antibiotic	Contagious (other cows)
Dry cow antibiotic	
Cull	

Sub-clinical mastitis is most commonly recognised by detecting the inflammatory process in the udder by testing a sample of milk. This can be using a simple but insensitive method such as California Mastitis Test (CMT) sometimes called Rapid Mastitis Test (RMT) which can give results within a few seconds and so is effectively a cow-side test but is semi quantitative giving results as negative or +, ++ or +++. There are many other methods of detecting sub-clinical mastitis with the developed dairy industries of the world most commonly using measurement of the number of somatic cells resulting in a Somatic Cell Count (SCC) for a milk sample.

b. What are SCCs?

The word Somatic is derived from the Greek word *somatikos* and means 'of the body'. SCC is measured in 1,000's cells/ml. Both the overall absolute number and proportion of the various types of leucocyte in milk will vary with a number of factors, including stage of lactation, time of year, diurnal variation, milking frequency and interval and stress, but the most significant changes are seen in response to infection. Increased SCC indicates an immune response most commonly to infection however it is also part of that immune response and confers some protection. Zero SCC at bulk tank, cow or quarter level is not achievable or desirable. Bulk Milk tank SCC indicates the prevalence of infection within a herd with an optimum level of around 100 to 150,000 cells/ml. Response to infection causes variation in absolute number and proportion of cell types. The majority of somatic cells in normal bovine milk are macrophages (65 to 85 percent), with other leucocytes, including lymphocytes (10 to 25 per cent) and neutrophils (0 to 10 per cent), making up the numbers, along with epithelial cells (0 to 5 percent). Milk from an infected gland, however, has in excess of 90 per cent neutrophils, with an unchanged proportion of epithelial cells and macrophages and lymphocytes making up the numbers. SCC is expressed as a number of somatic cells per ml of milk and can logically be used as an indirect measure or indicator of udder health (with the caveat that factors other than infection can affect SCC), because the majority of the SCC is made up of immune cells which are produced in greater numbers by an infected gland. For these reasons, changes in SCC are used as a proxy measure for infection. The implications and financial impact of increased SCC in terms of reduced yield and quality have never really been heeded by farmers, and it was only when the financial penalties became more direct by reduced milk value by way of a payment penalty that interest was focused on cell counts.

WHAT TYPE OF MILK CAN BE MEASURED?

a. Bulk milk somatic cell count (BMSCC)

A herd or bulk milk tank SCC is effectively made up of the SCCs of the individual cows contributing milk to the bulk tank at that time. It gives an estimation of how widespread infection is (prevalence) within those cows. Although the impact an individual cow has on the BMSCC will depend on a combination of her yield and the SCC, it can be estimated that there is an increase in prevalence of 10 per cent for every 100,000 cells/ml increase in the BMSCC. However BMSCC gives a poor indication of incidence as it is possible for a herd to have a low BMSCC with very high clinical mastitis rate. Also BMSCC can be farmer manipulated by keeping

milk from high SCC cows out of the bulk tank which reduces the value of the farm Bulk Milk SCC (only cows contributing to milk sold off farm) compared to the calculated or true milk recording BMSCC (all cows in milk) which gives a more realistic picture of the udder health status of the farm.

An illustration of the dangers of using bulk tank SCC to monitor herd status.

Herd 1
- 100 cow herd all with SCC = 200,000
  Bulk Tank SCC = 200,000

Herd 2
- 92 cows with SCC = 200,000
- 1 cow with SCC = 2,000,000
- 2 cows with SCC = 1,000,000
- 5 cows with SCC = 500,000
  Bulk Tank SCC = 250,000

b. Individual Cow Somatic Cell Count (ICSCC)

Regular monthly individual cow recording is probably the most common use of somatic cell counting and is almost exclusively performed on a composite commingled milk sample from all four quarters. This does introduce complications in interpreting the results as it represents the average of all four quarters (assuming all quarters are of equal yield). There is a danger that cows with infected quarters may go undetected, particularly if they have only one infected quarter with the other three having low SCCs.

An illustration of the dangers of interpreting composite cow SCC.

Comparison of quarter SCC for two cows with the same composite (comingled) cow SCC					
Likely infection status	Quarter cell counts				Composite Somatic Cell Count (cow)
	FL	FR	BL	BR	
Infected Cow	50	50	50	**450**	**150**
Uninfected Cow	150	150	150	150	**150**

USING ICSCC TO ASSIGN A LIKELY COW INFECTION STATUS

a. Detection tests vary in their ability to detect infection or absence of infection.

- CMT is relatively insensitive and can generally not detect SCC below 350 to 400,000 cells/ml and gives results in the form of negative, +, ++ or +++.
- Individual Cow SCC determination is most commonly performed on composite samples (comingled milk from all 4 quarters) and gives results in 1,000s cells/ml.

Unlike CMT SCC determination gives a number of somatic cells in a milk sample with varying degrees of repeatability depending on the range of the result with very low and very high results being less repeatable.

All diagnostic tests have inherent sensitivity (Sn), avoiding of false negatives [100 - false negative%] and specificity (Sp), avoiding of false positives [100 - false positive%].

b. Thresholds

A threshold is used to indicate infection status with results above the threshold allocated as likely infected and results below likely uninfected.

Internationally a threshold of 200,000 cells/ml is widely accepted and is useful for epidemiology as it gives a balanced Sn & Sp making similar “mistakes” in both directions which is useful when used on large datasets particularly when studying new infection and cure rates within a herd over time.

The use of a threshold of 200,000 cells/ml results in approximately a 75% sensitivity and 75% specificity but will vary with all the factors that impact SCC such as pathogen and stage of lactation but also the BMSCC of the herd ie prevalence of infection. Predictive values (Predictive +ve and predictive –ve) will vary from herd to herd and there will be a reduced sensitivity in higher BMSCC herds and a reduced specificity in lower BMSCC herds.

Effectively when used as a working threshold across many herds 75% of cows with an infection have a SCC >200,000 cells/ml and 75% of uninfected cows have a SCC <200,000 cells/ml.

CHANGING THE SCC THRESHOLD

DIFFERENT POPULATIONS JUSTIFY DIFFERENT THRESHOLDS.

a. Using lower threshold

This will improve sensitivity at the expense of reduced specificity Ie be more certain a negative result (i.e. a SCC below the threshold) is uninfected.

Maybe needed in:
- Younger cows e.g. heifers
- Herds with low BMSCC

Trade off between
↑ Sensitivity
and
↓ Specificity

Management of Somatic Cell Count (SCC)

Andrew Biggs

Also may be useful when:

- Running a clean group within a herd.

Using a lower threshold reduces the chance of infected cows being in the clean group although clean cows may end up in the infected group. This approach does preserve the clean group albeit at the potential expense of a few clean cows.

- Using a selective or targeted dry cow therapy protocol.

Dry period treatment cure rates are generally higher than lactation treatment cure rates and so all potentially infected cows need to be treated with antibiotic dry cow therapy at drying off. By using a lower threshold some uninfected cows may be treated with antibiotic DCT (although this is what happens to ALL cows in herds using blanket therapy).

b. Using higher thresholds

This will improve specificity at the expense of reduced sensitivity i.e. be more certain a positive result (i.e. a SCC above the threshold) is infected.

Maybe useful when:

- Selecting cows for sampling or treatment

Avoid wasting time and money sampling or treating cows which may not be infected with a major pathogen. However some infected cows could be missed.

Trade off between
↑ Sensitivity
and
↓ Specificity

WHAT CAN INDIVIDUAL COW SCC BE USED FOR?

Historically when ICSSC were introduced in the late 1980s, cows were just listed in descending SCC order resulting in a static snapshot approach as there was no attempt to assess changes to SCC dynamically.

More recently in the last 15 years, SCC analysis & management systems have been developed utilising computer software where dynamic correlation of changes of SCCs or more particularly change of infection status using most often a 200,000 cells/ml threshold gives an indication of infection dynamics during (a) lactation and (b) across the dry period.

a. Lactational Infection Dynamics

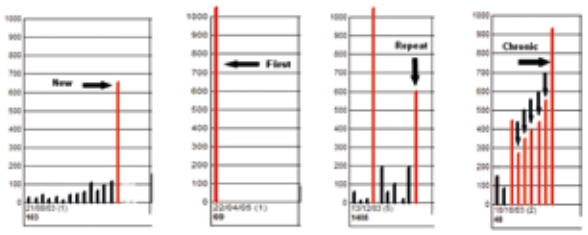
During lactation cows can be divided into 4 status categories of likely infected cows and 3 status categories of likely uninfected cows.

Likely infected status cows - SCC >200,000 cells /ml

- **New:** not the first milk recording in the lactation but the first with a high SCC Apparent New Sub-clinical Infection [ANSI].
- **First high:** both a high SCC and the first milk recording in a lactation.
- **Repeat:** a high SCC for at least the second time in the lactation, although following a low SCC at the previous milk recording.
- **Chronic:** a high SCC at both this and the previous milk recording.

Somatic cell count analysis - basic principles

Likely infected cows - text colour match rolling graph series colours.



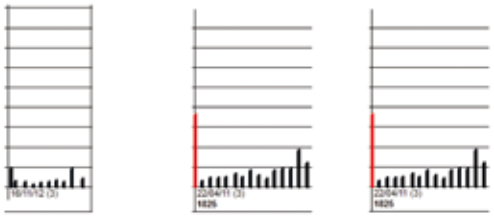
NEW FIRST REPEAT CHRONIC

Likely uninfected status cows - SCC <200,000 cells /ml.

- **First low:** both a low SCC and the first milk recording in a lactation.
- **Recovered:** a low SCC level following a high SCC.
- **Uninfected:** a low SCC at this and the previous milk recordings.

Somatic cell count analysis - basic principles

Likely un-infected cows - text colour match rolling graph series colours.



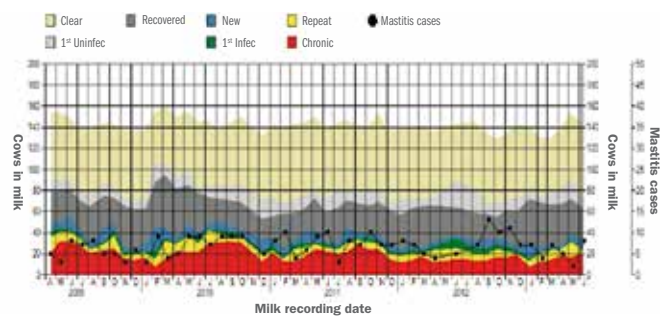
CLEAR CURE

a) From First a) From New

Graph of rolling data for SCCs for cows in lactation

The number of cows in each SCC categories are shown over time based on monthly SCC milk recording (DHI) data.

The black dotted line indicates clinical mastitis cases over time.



b. SCC dynamics during dry period

During the dry period cows can change their status in four ways (Using 200,000 cells/ml as a theshold to determine infection status).

LOW LOW: Uninfected cows remaining uninfected during the dry period. *(Target > 90% of cows drying off low i.e. dry period protection rate).*

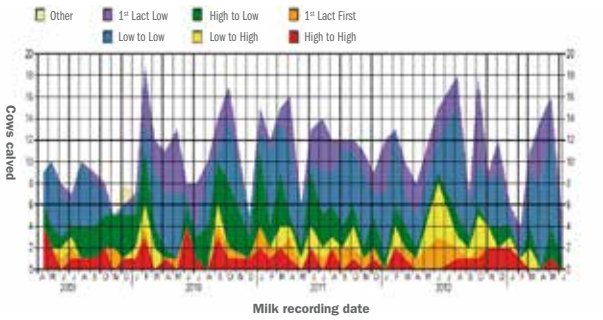
HIGH LOW: Cows eliminating an infection during the dry period. *(Target > 80% of cows drying off high i.e. dry period cure rate).*

LOW HIGH: Cows acquiring an infection during the dry period. Cows acquiring a new dry period infection are given a **First New** category.

HIGH HIGH: Cows remaining infected through the dry period. Cows failing to cure a dry period infection are given a **First Chronic** category.

Graph of rolling data for SCCs the dry period

The number of cows and heifers in each dry period SCC category are shown over time based on monthly SCC milk recording (DHI) data.



A summary of the performance of cows around the dry period

1. What % of cows end lactations with Low SCCs: Target >70%
2. How many heifers start lactations with Low SCCs: Target >90%
3. Dry period protection % (Low:Low): Target >90%
4. Dry period cure % (High:Low): Target >80%

The results for this example farm for each of the 4 parameters are circled.

Dry period performance for cows calving in the 300 days before 23/01/12											
Lactation	Lactation started	% low at end previous lactation		Start LOW	% start LOW		End LOW start LOW	% LOW:LOW		End HIGH start LOW	% HIGH:LOW
1	84			62	74%		0			0	
2	88	76%		72	82%		56	84%		16	76%
3	28	54%		25	89%		14	93%		11	85%
4	23	48%		20	87%		9	82%		11	92%
5+	22	27%		17	77%		6	100%		11	69%
total	245	61%		196	80%		85	86%		49	79%

REGULAR ANALYSIS AND INTERPRETATION OF MONTHLY MILK RECORDING DATA

Like fertility there are 2 sides to the approach.

a. Overview of herd performance

Like with fertility there are recognised performance targets:

PARAMETER	TARGET
BMSCC	< 150,000 cells/ml
New infection rate	< 5% cows in milk
Dry period new infection	< 10%
Dry period cure	> 80%
Proportion of herd > 200,000 cells/ml	<10%
Chronic rate	<5%

Where herd performance is worse than target in one or more parameter management changes can be implemented to address poor performance.

b. Action lists of cows needing attention

Again, like with fertility, individual cows can be identified as needing attention:

- Cows with recent infections during
  - Lactation
  - Dry period
- Cows with non-recovering new infections from previous month Chronic cows.

Cows potentially on action lists from routine monthly SCC records

- New infections
  - 1st time > 200,000 cell/ml this lactation.
  - Acquired a recent infection.
- First infections
  - Calved in > 200,000 cells/ml.
  - Acquired an infection in the dry dry period or failed to respond and eliminate an infection in the dry period.
- Non recovered recent infections
  - Last month's New or First infections still > 200,000 cells/ml.
  - Some repeat infections still > 200,000 cells/ml.
  - Some early chronic infections e.g. 3 consecutive high SCCs.

If an intervention is to take place on the above cows it will be either sample, treat or both sample then treat.
Improved specificity for intervention can be achieved by using a higher threshold eg 300,000 cells/ml.

Management of Somatic Cell Count (SCC)

Andrew Biggs

Cows potentially eligible to treat from routine monthly SCC records

News & Firsts. Although these have only one high SCC and many will self cure.

Non responding News or First from previous month. These are more significant in terms of being potential persistent infections which have the ability to become clinical or spread to other cows.

The decision to treat needs to take many factors into account:

- Herd factors
  - Economics and infection dynamics.
  - BMSCC and proximity to payment penalty threshold.
  - Increased or increasing contagious pattern new infection rate.
  - Dry period performance.

- Individual cow factors
  - Chance of cure
  - Pathogen
  - Strain (not currently commercially available but transmission patterns help identify potential contagious behaviour).
  - Chronicity (number of consecutive high SCCs).
  - Number of previous clinical cases.

Action lists from cows with high individual cow SCC (after interpretation and not just from one result)

Action for selected problem cows	Effect on cow and or herd
No action	Cow unaffected. Herd still at risk as infected quarter(s) still being milked through parlour $
Sample and culture	
Withhold milk (or feed to male calves)	
Treatment	Cow hopefully cured. Herd risk reduced / eliminated
Early dry off	Cow may cure. Herd risk reduced / eliminated #
Quarter dry off / quarter culling	
Cull cow	Cow removed and herd risk eliminated #

$ BMSCC will be reduced by "manipulation" as the high SCC milk is not entering the tank. However the risk of spread is still present although some form of cluster disinfection or milking last would help.

Often by the time chronically infected cows are identified, and eventually culled, spread to other cows within the herd will have already occurred. So even though, as far as the herd is concerned, culling will effectively remove the infection in that cow infection will often be established in other cows which in time may well become chronic high cell count cows themselves.

HERD LEVEL DIAGNOSIS

The effective text book characterisation of bacteria as Contagious / Environmental is no longer sufficient. Characterisation resulting in a Herd level diagnosis of Contagious / Environmental and Lactation origin / Dry period origin is more appropriate.

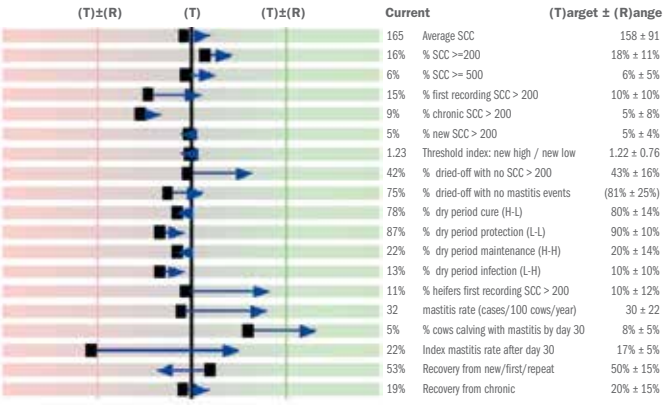
The diagnosis will be influenced by:
Prevalence of potential mastitis pathogens isolated.
Transmission patterns shown by data.
Management / risk assessment profile.

So let's see how we make the diagnosis

1. Look at an overview of the data Lactation origin or Dry period origin?
2. Look in detail at transmission patterns - Contagious or Environmental?

The next section uses graphics from Interherd plus PAN Livestock Services Ltd. There are many similar software packages around the world however the graphics are used as examples of how SCC data can be analysed diagnostically.

Key Performance Indicator (KPI)



The value to the left of each parameter is the herd performance over the last 12 months and is represented by the black square with the blue arrow indicating the direction and magnitude of change in the last 3 months.
The vertical black line represents the target for each parameter which is achieved by the best 25% of a cohort of National Mastitis Records (NMR) DHI recorded herds.
For any parameter if the black square or tip of the blue arrow is
- To the right of the vertical black line the herd is in the top 25% of herds for that parameter.
- To the left of the pink line the herd is in the bottom 25% of herds for that parameter.

ORIGIN CHARACTERISTICS

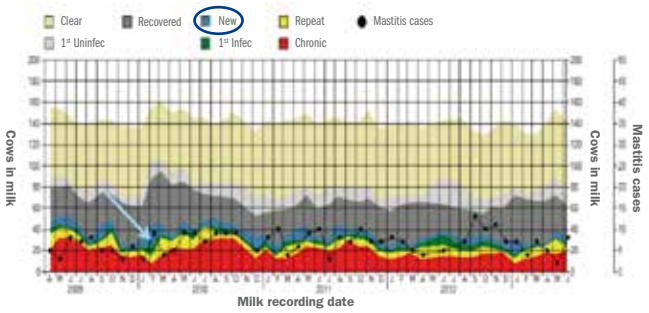
a. Lactation origin characteristics

Can be either environmental or contagious pathogens and transmission patterns.

- >17% index clinical cases in cows more than 30 days calved (2 in 12 cows calving in analysis period). Index case is the first clinical mastitis case in the current lactation.

- >5% new IMI's based on increase from below 200,000 cells per ml threshold to above threshold at DHI recording ("News") other than first DHI recording within 30 days of calving ("Firsts").

Lactation origin > %5 New IMIs



b. Dry period origin characteristics

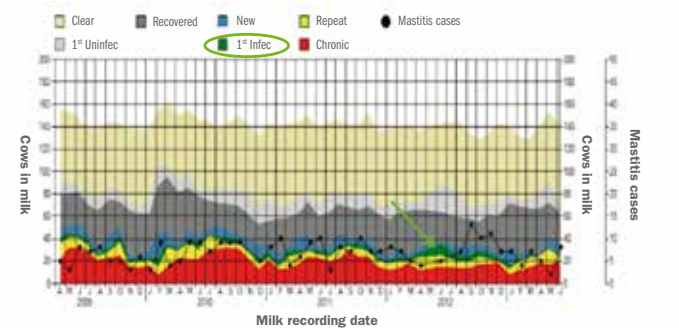
Most commonly environmental pathogen and transmission patterns. Cows are not being milked to facilitate contagious spread.

- High incidence of new IMIs in early lactation.

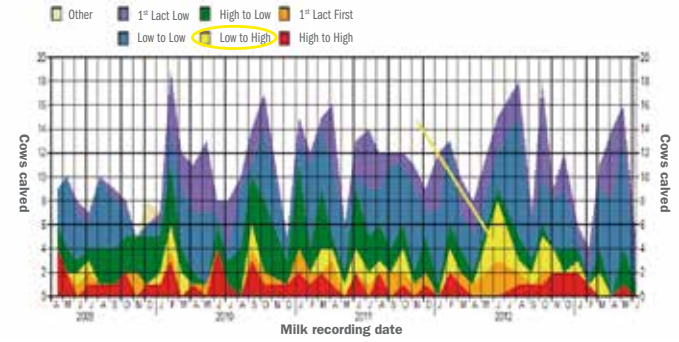
- >8% index clinical cases within 30 days of calving (1 in 12 cows calving in analysis period).

- >5% cows with failure of dry period protection (Low:High).

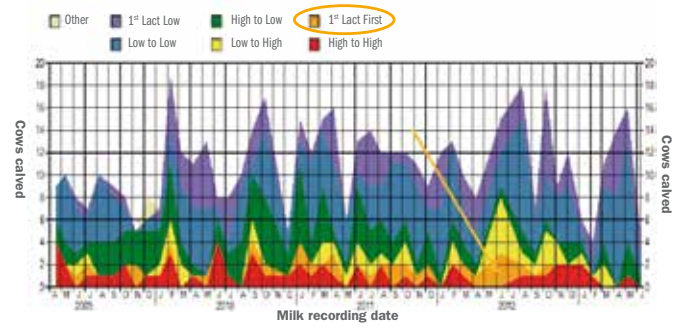
First infection
Cows freshening (first SCC of lactation) with a high SCC. It is made up of failed dry cure (High:High) and failed dry protection (Low:High) – Overall target < 10%.



Dry period origin characteristics >5% COWS (parity 1 or greater) with failure of dry period protection (Low:High).



Heifer "dry" origin new infection >5% heifers with failure of "dry period" protection (High at 1st recording).



So what do we tell farmers to NEVER do with individual cow SCCs?
NEVER assign an infection status from ONE SCC!

So what do we do with dry period performance?
We use:
ONE SCC before dry off (H or L).
ONE SCC after calving (H or L).

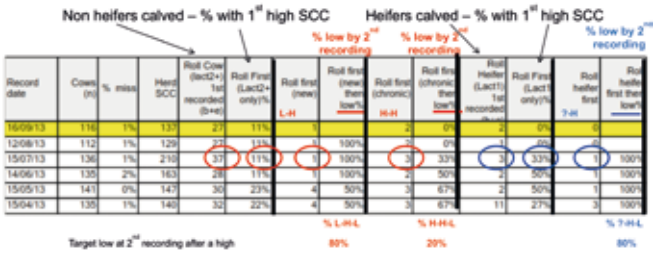
So what about the 2nd recording after calving?
Maybe it is a transient high SCC after calving.

Management of Somatic Cell Count (SCC)

Andrew Biggs

What happens to cows & heifers that have a 1st recording > 200,000 cell PER ML DO THEY STAY HIGH?
What proportion are low by their second recording?
SCC analysis by recording date displaying rolling 6 month data as numbers of cows are often low.

The table shows details of cows and heifers 1st recorded with First (high) rates and proportions that recover by 2nd recording for 1. First (new), 2. First (chronic) and 3. Heifers.



TARGETS
1. Column 8. First new recovery % (LHL) 80%.
2. Column 10. First chronic recovery % (HHL) 20%.
3. Last column. Heifer first recovery % (?HL) 80%.

Herds with a high prevalence of CNS or *Corynebacterium bovis* around calving are likely to have more cows Low at 2nd recording than herds with a high prevalence of *Staph. aureus*.

TRANSMISSION CHARACTERISTICS

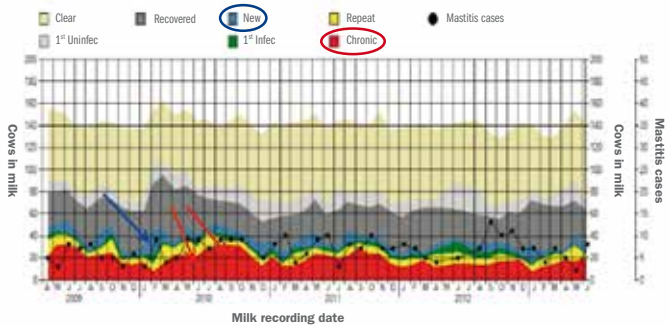
a. Contagious transmission characteristics

- Long duration of intramammary infections (IMI's).
- Relatively high BMSCC.
- Multiple clinical episodes from a single quarter – (recurrent cases).
- High cow SCC in DHI data in months before clinical episodes.
- Strong link between the prevalent existing infections and likelihood of new infections with the same pathogen.
- Positive correlation between the prevalence of existing infections (% chronic high SCC cows) and risk of new infections (% new high SCC cows).

Looking at lactation origin - new infection again

>5% NEW IMIs based on individual cow SCC defines it as Lactation origin however. What is the relationship between NEWS and CHRONICS.

- If there is a positive correlation as shown in the graphic by the new infections increasing (blue) and the chronics increasing in subsequent months (red) this is likely Lactation contagious
- If there is no correlation or a negative correlation lactation environmental is more likely.



b. Environmental transmission characteristics

- Relatively short duration of IMI's.
- Potentially Low BMSCC.
- High incidence of clinical cases without presence of contemporaneous long duration IMI's.
- Low cow SCC in DHI data in months before clinical episodes.
- High incidence of peripartum IMI's and clinical cases.
- No link between the prevalent existing infections and likelihood of new infections with the same pathogen.
- Negative or no correlation between the prevalence of existing infections (% chronic high SCC cows) and risk of new infections (% new high SCC cows).

FINAL HERD DIAGNOSIS

Using the above data analysis and pattern recognition we come to the main issue for the herd as being predominantly one of the following possible herd diagnosis:

- Dry period Environmental
- Dry period Contagious

- Lactation Environmental
- Lactation Contagious

OTHER MEASURES TO BE MONITORED

a. Clinical mastitis cases and ICSCC

Monitor SCC prior to index clinical mastitis cases.

- Numbers circled in green are the number of likely UNinfected cows.
- Numbers in red squares are the number of likely INfected cows

Filter: Days PP >30; Qu cases New 1

Prior SCC type / No. observations							
1st Uninfected	Clear	Recovered	First (new)	New	Repeat	Chronic	
(3)	(14)	(9)	[2]	[4]	[2]	[5]	Overall

Monitor SCC subsequent to index clinical mastitis cases.

Filter: Days PP >30; Qu cases New 1

SCC Outcome @14-44 days / No. observations				
Low	High	Dry	Not milked	
(13)	[10]	6	10	Overall

If either SCC prior or subsequent to index clinical cases (not repeat clinical cases) is high it might imply contagious components to the epidemiology and/or poor cure rates.

But remember:

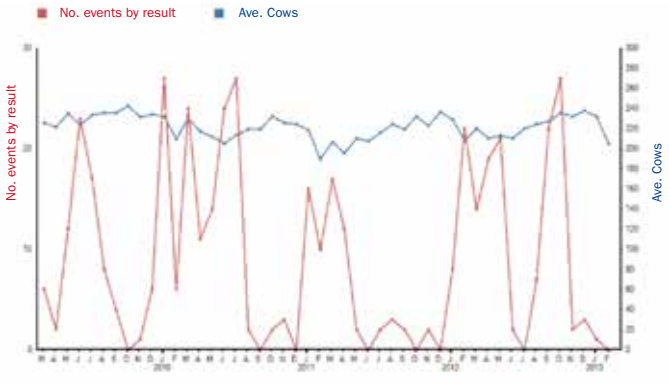
- Dry period origin is likely if index clinical cases are predominantly in cows < 30 days DIM.
- Lactation origin is likely if index clinical cases are predominantly in cows > 30 days DIM.

b. Age profile of various SCC types

The table below shows a normal age profile with more older cows being infected.

Rolling 3 month data by lactational age.

SCC status summary from 29/03/15 to 29/06/15												
Parity	Total milk tests	New	First infected	Repeat	Chronic	**Total infected**	% infected	Recovered	First uninfectec	Uninfected	**Total uninfected**	First Infection %
1	254	2	0	2	4	**8**	3%	52	24	170	**246**	0%
2	202	6	1	2	14	**23**	11%	42	13	124	**179**	7%
3+	354	16	2	8	48	**74**	21%	48	28	204	**280**	7%
Total	810	24	3	12	66	**105**	13%	142	65	498	**705**	4%



Seasonality tends to suggest environmental influence
Beware if herd has very seasonal calving pattern!

WHAT WE HAVE LEARNT

- What SCCs are and what they mean.

- How we have got smarter:
  - In how we analyse SCCs?
  - How we audit herd farm records for mastitis?
  - How we make a final herd diagnosis?

Management of Somatic Cell Count (SCC)

Andrew Biggs

WHAT DO YOU KNOW ABOUT...

1. What cells are most common in an uninfected mammary gland?
a. Lymphocytes
b. Neutrophils
c. Macrophages
d. Epithelial cells

2. What cells are most common in an infected mammary gland?
a. Lymphocytes
b. Neutrophils
c. Macrophages
d. Epithelial cells

3. What is the internationally accepted "balanced" SCC threshold that used for differentiating infected from uninfected cows?
a. 100,000 cells per ml
b. 150,000 cells per ml
c. 200,000 cells per ml
d. 250,000 cells per ml

4. What is the SCC detection threshold for CMT
a. 400,000 cells per ml
b. 300,000 cells per ml
c. 200,000 cells per ml
d. 100,000 cells per ml

5. If you want to be more certain a cow is Uninfected for example to use selected or targeted dry cow therapy how would you modify the SCC threshold you use?
a. Use a higher threshold than the standard 200,000 cells per ml.
b. Use a lower threshold than the standard 200,000 cells per ml.

6. What is the classification for a cow that has a rise in SCC from below to above the threshold between successive monthly milk recording (DHI) results?
a. First
b. New
c. Repeat
d. Chronic

7. Using monthly DHI data what is the target percentage of clean (low and below SCC threshold) cows at drying off that should be clean (low and below SCC threshold) after calving?
a. 60%
b. 70%
c. 80%
d. 90%

8. Using monthly SCC (DHI) data what is the likely transmission pattern of mastitis infection if after an increase in New infections results in an increase Chronic infections in subsequent months?
a. Contagious
b. Environmental

9. What is the target percentage of new dry period infections (L-H) that should self-cure (L-H-L)?
a. 20%
b. 80%
c. 70%
d. 50%

10. Which one of the following characteristics only fits with environmental origin mastitis?
a. >5% new IMI's based on increase from below 200,000 cells per ml threshold to above threshold at DHI recording ("News") other than first DHI recording within 30 days of calving ("Firsts").
b. Long duration of intramammary infections (IMI's).
c. High incidence of periparturent IMI's ("Firsts") and index clinical cases in cows less than 30 days calved.
d. High cow SCC in DHI data in months before clinical episodes.

Find the answers below:

1c, 2b, 3c, 4a, 5b, 6b, 7d, 8a, 9b, 10c

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How to R.E.S.E.T. a farmers mastitis mindset?

INTRODUCTION

Historically agriculture was assumed to be an activity executed by an individual farmer based on rational, technical, and economic considerations[1, 2]. Of course, ultimately these factors are crucial as a base for making sound decisions. For farmers, however, decision making about mastitis management is not always that rational and seems far from clear and understandable[3]. It is unclear, why farmers, although it would benefit their results, do not implement effective mastitis management practices[4]. Over the years it became clear that, besides deliberate rational considerations, farmer mindset factors play an important role in decision making[1, 3-14]. This paper describes the R.E.S.E.T model that can be used as a framework for changing the farmer's mindset towards mastitis, resulting in an improved udder health.

FARMER MINDSET

The mindset of the farmer, as any other individual, comprises many social psychology constructs such as personality, knowledge, intentions, attitudes, beliefs, values, skills, perceived norms, and perceived self-efficacy. This mixture of influences leads to a certain behaviour, generally summarized as 'mindset', and has been described in several models, such as the Theory of Planned Behaviour[15-17] and the Health Belief Model[18-20]. Both are frequently used to explain people's health behaviour[21-23].

The results of several studies of the Dutch Udder Health Centre, have shown that behaviour towards mastitis can be explained by two important determinants, being the perceived threat ("Do I have a problem?") and the perceived efficacy of potential approach of the problem ("Can I solve the problem?")[24, 25], factors we know from the Health Belief Model, that is presented in Figure 1. [18-20, 26, 27]. It is important to acknowledge these factors and to make sure to have an understanding about farmer's perceptions on benefits and barriers of preventive measures when advising them.

Research has shown that mastitis incidence indeed can be explained – to a certain extent – by farmer mindset[28, 29]. In these studies, elements of farmer mindset explain 17% of the variance in clinical mastitis incidence and 47% of the variance in bulk milk somatic cell count (BMSCC), while farmers' self-reported behaviour explains, 12% respectively 14% of the variance of these parameters.

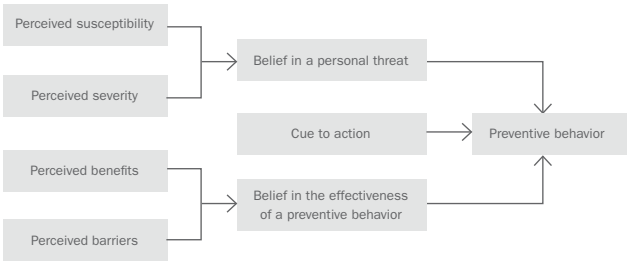

Figure 1. The Health Belief Model [18], adapted by Koelen and Van den Ban[30].

THE R.E.S.E.T APPROACH

In order to improve udder health, mastitis management has to be optimised. The most important part of mastitis management is the behaviour of the farmer which, is mainly influenced by mindset. Luckily, there are many ways to persuade farmers to change their behaviour regarding udder health[25-27, 31-33]. Crucial, however, is to note that farmers are different. They have different learning styles and one is most successful in chancing behaviour, when differences between farmers are appreciated through customized communication[32, 33]. Hence, in order to reach large numbers of farmers, combinations of actions and strategies are needed.

Theoretically an analysis and individually adapted approach of every farmer would work best. In daily practice, however, this is impossible. Luckily, some successful intervention strategies are available, to make life easier. One of them is the R.E.S.E.T model, that was adapted from van Woerkum et al.[34], and Leeuwis[1], and has been used in a slightly different form for mastitis before[33]. Basically, the model uses five instruments to change behaviour of people: The R of Regulations, the E of Education, the S of Social pressure, the E of Economics, and the T of Tools. Because people are different, and are sensitive to different stimuli, this mixture of approaches makes a program effective[26].

The R.E.S.E.T. model includes a voluntary as well as a compulsory approach. The compulsory approach consists of coercion such as regulations and restrictive provisions[34, 35]. Compulsory change of behaviour, however, will probably only last as long as the coercion exists. Voluntary behavioural change therefore is preferable. This can be reached by internal or external motivation of which the former is most effective, but the most difficult one

How to R.E.S.E.T. a farmers mastitis mindset?

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to reach. External motivation is easiest to influence and, to our opinion, highly underestimated towards its possibility to change mastitis management behaviour.

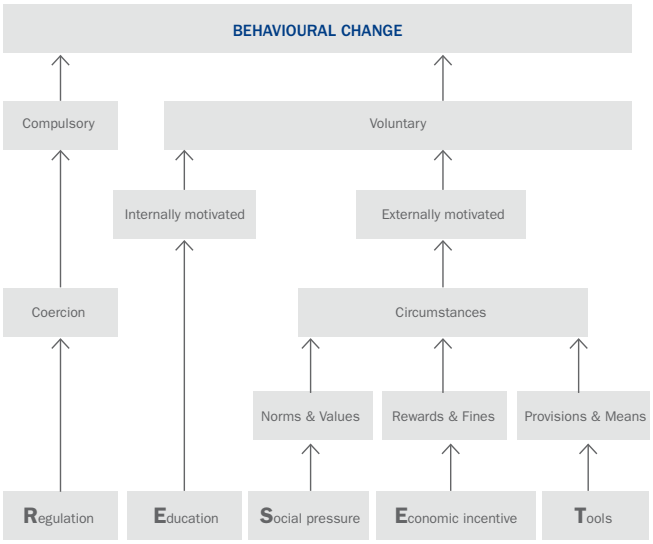

Figure 2. The R.E.S.E.T model: Behavioural change by a combination of strategies (adapted from Van Woerkum et al.[34], see also Leeuwis[1]).

THE R OF REGULATIONS: FOR CLARITY SEEKERS

Of course regulations stand for rules that force people to behave in a certain way. If you don't, you can end up with a fine or worse. This approach is based on coercion and has a well-known effect with respect to BMSCC and residues of antibiotics and other drugs. Prerequisite for success, of course, is a proper surveillance system.

Another aspect of rules, is that they can bring clarity to those seeking for structure, the so-called "clarity seekers". These people are not interested in 'why', they just want to know 'how'. Clear directions can be very helpful to them.

THE E OF EDUCATION: FOR INFORMATION SEEKERS

Probably the most used intervention instrument is education. Although the effect of technical knowledge is beyond any doubt, the effect of education may, at times, be overestimated. The most important reason for that, is that education often is approached from the rational, technical perspective. Just sending information, irrespective how good it is, will have a limited effect. Only internally motivated people will pick it up[31].

Education will be most effective if information is offered in different ways to people with different learning styles[33]. If you do take differences between people into account, even farmers that at first sight seem hard to reach, can be influenced. Farmers for instance, who know everything better that you do, the so-called Dr. Googles, can be triggered by openly appreciating their knowledge, followed by a discussion on scientific information from a variety of sources. Another example is the use of study groups, which is quite effective for a specific group of internally motivated farmers with a specific learning style[31, 33, 36].

THE S OF SOCIAL PRESSURE: FOR STATUS SEEKERS

Because social pressure influences norms and values, it can also affect internal motivation. Social pressure influences people's frame of reference and determines what is considered normal on a farm. As such, social pressure often works through circumstances on a farm or in a family. Because humans cannot function without social cohesion, social pressure is one of the most powerful tools that can be used to change behaviour. The effect of study groups where farmers influence each other, likely is based on that principle. In a conversation with an individual 'status seeker' you can benchmark the farm data with other farms, for instance with the national average, to stimulate them to change behaviour in order to improve results.

The more people within a farmer's social network put pressure on a farmer, the harder it is not to comply. That's why it is important that all vets within a practice, and preferably all advisors coming on a farm, cooperate and send the same message. If the social pressure is high enough the other tools of the R.E.S.E.T model may have only a limited effect.

THE E OF ECONOMIC INCENTIVES: FOR PRICE SEEKERS

External motivation can, amongst others, be accomplished by financial stimuli such as bonuses and penalties related to BMSCC[37, 38]. In many countries a penalty is imposed for a geometric mean BMSCC above 400,000 cells/ml. The efficacy of that approach has been experienced over and over, reducing the number of herds with a BMSCC above the threshold level chosen. BMSCC penalties, however, do not solve udder health problems such as clinical mastitis, because serious clinical mastitis problems may also occur in herds with a low BMSCC[39, 40]. Interestingly, penalties not only work through coercion, they also have an effect on the social norm, because they tell you when you are not doing well.

A consequence of setting levels that will be penalized, is that some farmers are satisfied with a BMSCC below that level, while in fact you would want them to be more ambitious. Hence, it would be wise to think of a system that rewards farmers with lower BMSCC, as is done is some countries, by paying premiums for milk <250.0000 or lower. Here also, social pressure has its effect. It is not only the financial incentive that is rewarding, but receiving a premium for high quality milk, touches on the farmer's sense of pride and status. As other people, farmers like to have the feeling they are good at what they are doing, having best milk quality, lowest cell count, best udder health etc., and they like to show it.

Of course, economics can also have an effect through prizing of products. If certain products or services are offered in a financial attractive way, that may motivate some farmers, specifically the so-called price seekers to make use of them.

Apart from penalties, incentives and discounts, economic incentives can also work via increased profit due to better udder health. By improving udder health management, costs can be decreased and profits increased. Visualizing these effects may help in convincing farmers to change their behaviour. It should, however, be taken into account that most farmers do not behave economically rational[38, 41-44].

THE T OF TOOLS: FOR CONVENIENCE SEEKERS

There are many tools available to optimize udder health, being technical provisions, as well as several means and methods, such as analytical software. Tools can make a certain desired behaviour easier to perform, and as such is part of the Health Belief Model (figure 1).

Tools may take away barriers for people to change behaviour, that would otherwise not do so. In a conversations with these convenience seekers, you can point this out and stimulate them to take little steps towards change, for instance by doing an analysis of year-round mastitis data, rather than aiming at a monthly approach. Technical provisions can also be a big help in chancing behaviour. Sometimes simple adaptations, like the amount of light in the milking parlour, can hugely influence behaviour like the early diagnosis of clinical mastitis cases.

Tools do not necessarily have to be used with the goal to improve udder health. Milking gloves, for example, can be used for reasons of hand hygiene rather than udder health, but can still have their effect. Scientists are more and more aware of the effect of that type of automatic unconscious behaviour in daily life. With our growing capacity to analyse peoples brains we get a better picture of what happens within the unconscious brain.

THE MASTITIS VACCINATION EXAMPLE

Imagine a dairy farm which has had clinical mastitis problems for many years. All imaginable preventive measures have been taken. These measures were partly successful, but there still are *E. coli* problems, which sometimes lead to cases of toxic mastitis. The farmer is still not happy. You, as the farmers herd health advisor, think that vaccination is a possible solution to the problems. In order to convince the farmer to introduce this new management measure you can use several buttons of the R.E.S.E.T. model. Using the R of Rules, you can stress the disadvantages and undesirability of the excessive use of antibiotics and provide clear vaccination schedules and instruction. Applying the E of education you can show scientific papers to rationalize the added value of mastitis vaccination and discuss the principles behind it. The S of Social pressure can be applied by showing positive experiences of other farmers and you may connect farmers with each other to share experiences, or google together on reviews of the product. You can also benchmark farm characteristics with other farmers in the region and show how they deviate. You can press the Economics button by calculating the positive effect mastitis vaccination may have on this farm, showing how much each case of clinical mastitis costs and calculate the Return On Investment. Or you could give free trials (no cure no pay) or provide added value such as free services like taking milk samples for bacteriological culturing for free. By pressing the Tools button you will make life easier for the convenience-seeking farmer. It is clear that vaccination on itself is a tool already, to increase resistance of cows. You can also provide for example an indestructible vaccination scheme and reminder stickers and show farmers how easy it's done. Provide proper gear and ready to use materials and take care of a quick distribution of the product. Measure performance and celebrate success.

CONCLUDING REMARKS

The farmer's mindset is crucial in mastitis control. Farmers' behaviour towards mastitis management is influenced by determinants such as perceived threat ("Do I have a problem, and how serious is that problem?") and the perceived efficacy of preventive measures ("Is there a solution, and how easy is it to execute that solution?"). This type of determinants should be addressed in strategies using the R.E.S.E.T model to change behaviour. The R.E.S.E.T. model can be used as a guide to evaluate communication strategies applied by veterinary practices and other advisors involved in udder health programs. The first prerequisite of a mastitis program is that its technical information about best management practices is up to standards. To be efficacious, however, it also should have a customized communication strategy

How to R.E.S.E.T. a farmers mastitis mindset?

Jolanda Jansen, Roeland Wessels, Theo Lam

with an integrated approach. Such programs need to be supported by all stakeholders involved, combining several policies, because farmers are part of, and are influenced by, a wide institutional context.

TEN QUESTIONS TO THINK ABOUT:

1. What different types of farmers exist?
There are many different ways to segment farmers, e.g. based on demographics such as education level, age, income or type of farm. You can also segment your clients based on personal characteristics, for example whether they are extrovert or introvert or whether they like or reject digital technology or you can segment them based on their importance for your business, e.g. loyal clients versus critical clients or large versus small turnover. In this chapter we distinguish five types: clarity seekers, information seekers, status seekers, price seekers and wait-and-see-ers. More important than how you segment them, is that you do segment them. Segmentation of clients helps you to tailor your communication to the needs of specific groups.

2. What is your own preferred communication style, does it fit with all your farmers?
Understanding your own competences and communication style is key to effective communication with clients. If you are not on the same level, it will be difficult to understand and motivate the client. Then you have two options: adapt your communication style to the farmer, or ask a colleague with a better matching communication style to advise the farmer.

3. What are the main drivers for change?
There are many drivers for change: internal motivations and external circumstances. Basically it comes down to two things: Does the farmer perceive a problem (what's the chance for the problem to happen and how severe are the consequences) and Does the farmer perceive effective solutions (what are benefits and barriers for solving the problem).

4. Which five 'buttons' can you push to change farmer behaviour?
R.E.S.E.T. Rules and regulations, Education, Social pressure, Economic incentives and Tools.

5. Why is the educational route to change behaviour not effective for all farmers?
This refers back to question 1. Different types of farmers exist. Not all farmers have the same learning style. Some prefer to be educated, some others prefer to experiment and search for solutions themselves. We know that most decisions are rationalized afterwards, while at the moment they are taken, decisions are based on all kinds of unconscious internal deliberations and often are driven by external cues. Thus, you can provide a huge amount of scientific arguments, but even though the farmer believes you are right that does not necessarily mean he is going to change. To change routine behaviour you may have to push other R.E.S.E.T. buttons.

6. How could you persuade a farmer by using economic arguments?
When showing economics it is important to distinguish between short- and long term costs and benefits. Know your figures. An average case of clinical mastitis cost € X , if you prevent Y cases of mastitis with vaccination you save € Z.

7. When using social pressure, who should you involve related to farm management decisions?
For each farmer this is different. Know your farmers: who are important to them? Who is most influential? With whom are they talking about farm management? This could be the partner, employees, nutrition specialists, the accountant, hoof trimmers, equipment suppliers etcetera. It is important for each situation to assess this so-called social network, which is topic specific. For mastitis they may have a different social network than for breeding strategies or claw health.

8. How could you benchmark farm performance indicators?
For farmers, and people in general, it is important to benchmark yourself against others. What is the social norm? What is acceptable or not? Try to make this visible within your region or on national level. It depends on the subject how to actually quantify farm performance. Sometimes hard data are available, i.e. milk production or somatic cell count. In that case you only have to visualise the data and compare the specific herd to others. In other situations no data are available or maybe hard to quantify, the so-called soft data. These data have to be collected pro-actively by asking questions. There are several options in between these two extremes.

9. What kind of questions should you ask a farmer to determine his mastitis mindset?
Ask open-ended questions without being suggestive. Ask and listen to the farmers reply without judgement. What are his goals? What will his farm look like in 5 years from now? What are his biggest worries? Where does he find information? Who supports the farmer in taking decisions? What does he want to achieve on the short and on the long term? What are priorities? What does he perceive as a problem and when is he satisfied with a solution? For this kind of questions it is important that the balance in the conversation between farmer and advisor is at least 80%-20%.

10. How do you train yourself in communicating with different types of farmers?

Although challenging, reflection on your own behaviour is the key to communication. Miscommunication is a sender-error. If you have told it the farmer already for the tenth time and he still doesn't comply, you had nine opportunities to do it different. It starts with knowing yourself and your strengths and pitfalls. Ask colleagues, friends and clients about the way you communicate. Use for example the 360 degrees feedback method. Knowing your pitfalls you can train yourself.

Role-playing games is not liked by many, but it is a very powerful instrument to learn to control and adapt your primary reactions in communication in a safe environment. Both verbal and nonverbal. Always use a positive feedback method: First a TOP, than a TIP, or first a compliment, than an advise. Intervision with your colleagues is very helpful as well. You may join each other when visiting farmers. Give each other feedback and learn from best- and worst cases. Set a target for yourself, using small steps, e.g. at least two open-ended questions in every conversation. Ask yourself after every visit whether you have met your own target and how the farmer responded to that.

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How to R.E.S.E.T. a farmers mastitis mindset?

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STARTVAC® , Polyvalent inactivated vaccine, bovine mastitis, in injectable emulsion. **COMPOSITION:** *Escherichia coli* (J5) inactivated > 50 RED_{80}* *Staphylococcus aureus* (CP8) strain SP 140 inactivated, expressing Slime Associated Antigenic Complex (SAAC) > 50 RED_{80}** * RED_{80}: Rabbit effective dose in 60 % of the animals (serology). ** RED_{80}: Rabbit effective dose in 80 % of the animals (serology). **INDICATIONS:** For use in healthy cows and heifers, in dairy cattle herds with recurring mastitis problems, to reduce the incidence and the severity of the signs of clinical or sub-clinical mastitis caused by *Staphylococcus aureus*, coliforms or coagulase-negative staphylococci. **ADMINISTRATION ROUTE:** Intramuscular use. The vaccinations should be preferably administered on the alternate sides of the neck. **DOSAGE:** Administer one dose (2 ml) by deep intramuscular injection in the neck muscles at 45 days before the expected parturition date and 1 month thereafter administer a second dose (at least 10 days before calving). A third dose should be administered 2 months thereafter. The full immunization program should be repeated with each gestation. **SIDE EFFECTS & CONTRAINDICATIONS:** Adverse reactions: Slight to moderate transient local reactions may occur after the administration of one dose of vaccine. They would mainly be: swelling (up to 5 cm^2 on average), which disappears within 1 or 2 weeks at most. In some cases, there may also be pain at the inoculation site that spontaneously subsides in a maximum of 4 days. Animals vaccinated with an overdose did not show adverse reactions other than those observed after the administration of one dose of vaccine. **CONTRAINDICATIONS:** None. **WITHDRAWAL PERIOD:** Milk: None. **SPECIAL PRECAUTIONS:** Only healthy animals should be vaccinated. Allow the vaccine to reach a temperature of +15 °C to +25 °C before administration. Shake before use. **SPECIAL PRECAUTIONS FOR THE PERSON ADMINISTERING THE MEDICAMENT:** This product contains mineral oil. Accidental injection/self injection may result in severe pain and swelling, particularly if injected into a joint or finger, and in rare cases could result in the loss of the affected finger if prompt medical attention is not given. Can be used during pregnancy and lactation. Store and transport refrigerated (+2 °C to +8 °C) and protected from light. Do not freeze. Further information available from SPC. **PACKAGING:** Cardboard box with 20 vials of 1 dose. Cardboard box with 1 vial of 5 doses. Cardboard box with 1 vial of 25 doses. Under veterinary prescription. **MARKETING AUTHORIZATION NUMBERS:** EU/2/08/092/003; EU/2/08/092/004; 2/08/092/006. **MARKETING AUTHORISATION HOLDER:** LABORATORIOS HIPRA, S.A. Avda. la Selva, 135. 17170 Amer (Girona) Spain. Tel. (972) 430660 – Fax (972) 430661. **Use medicines responsibly.**

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