

Managing Mastitis 7

Collecting quarter milk samples for analysis

The individual quarter milk analysis allows the identification of micro-organisms that are causing a specific case of mastitis. In a second step, the performance of an antibiogram allows the identification of the most suitable, means effective, antibiotic treatment.

Aseptic sampling

Since the selection of such an appropriate antibiotic treatment will be determined by the results of the milk sample analysis, the collection of quarter samples has to be done in an aseptic way in order to avoid any contamination. Therefore, it is recommended that sampling is done by a veterinarian or another qualified person.

In order to assure the reliability of the analytical results, the following rules should be applied in the collection of samples:

At the site of sampling anything is to be avoided that could contaminate the sample, for example dirty hands, draught, handling of powdery substances, silage etc.

The sample must be collected either before milking or no earlier than three hours after the last milking, but should not be done beyond six hours after the last milking in order not to initiate premature milk release.

Free from drugs

The collection needs to be done prior to the administration of any drug. In case the cow has been treated before with an antibiotic, you have to wait 8 to 10 days

after the last application of the antibiotic.

Prior to sampling all organic material needs to be removed from the teat. For this purpose disposable (single use) paper is to be used or a moistened cloth (if possible using also water and soap).

Subsequently, you have to disinfect the teat, especially the sphincter, with cotton or gauze soaked with alcohol.

Before collecting a sample the foremilk must be discarded into a container (never on the floor). Subsequently the sterile tube is opened in a horizontal position.

The stopper should be held with the opening towards the floor in the same hand holding the tube to avoid contamination.

Collecting of the sample has to be done in a single stroke towards the tube walls. One millilitre will be enough.

Keep quarters separate

The milk from different quarters must not be mixed. Only if the analysis for contagious pathogens is the objective, mixing of quarter samples is appropriate. Preservatives must not be added in any case.

The samples should be individually numbered to allow for identification. They should also carry the number of the cow, quarter affected, date of sampling and the name of the person responsible for sampling.

The samples need to be stored at 4°C if it is shipped within the first 48 hours to the laboratory. If it needs to be stored longer, it needs to be frozen.

Courtesy of: www.solomamitis.com



Managing Mastitis 8

Control in heifers

Without doubt, young replacement stocks and heifers justify a maximum of care and attention as they represent tomorrow's milking cows and hence are key to the future income from dairying.

Mistakes made in raising heifers may often go unnoticed as no immediate consequences are seen. However, they will be paid for duly in the subsequent lactation in terms of disease and performance loss.

Furthermore, certain problems which are immediately noticed in dairy herds, often are – although present – not recognised in heifers kept in more remote areas.

One of those problems is mastitis in heifers. Estimates say that heifers are infected before calving to a level of up to 50%. Studies show that heifers infected pre-calving develop clinical mastitis in almost 80% of all cases after freshening, and milk performance fails to reach the level of healthy heifers by as much as 15%. Worst of all, milk performance even in subsequent lactations is unlikely to get fully restored, and Somatic Cell Count (SCC) may always be at risk of exceeding threshold levels for healthy milk.

Bacteria most predominant in causing heifer mastitis are coagulase negative Staphylococci, followed by Staph. aureus and Streptococcus uberis.

Among factors found to contribute to a higher risk of infection are high herd SCC, fly overload, feeding mastitic milk to calves, suckling between calves, contact to adult cows, poor housing (humidity, hygiene), milk leakage and oedema of udder or teats, and age at first calving (the percentage of heifers with a SCC of >200,000 increased from 22 to 27%, when calving age went up from 25 to 30 months).

How to detect infection

An infected heifer not showing any signs of mastitis is almost impossible to detect unless udder secrete is collected. Taking a sample without any evidence of an infection, however, may do more harm than good, as the sample collection requires the natural teat closure to be broken, which acts as a barrier to environmental pathogens.

Rather, all heifers should be care-

fully investigated about 2-4 weeks before calving for visible or palpable signs of mastitis. If done by trained personnel, studies show a high success rate in detecting mastitic heifers – 8 out of 10 quarters diagnosed with mastitis in fact came down with clinical mastitis in the subsequent lactation. In case of any doubts, antibiotic treatment is advised.

How to treat a heifer

Two key questions arise: when to treat (which is partly answered by the date of investigating the animal) and what to treat with. The earlier the analysis the less likely is it to detect an infected animal. The multiplication of pathogens and thus, the formation of visible or palpable symptoms are fostered by the presence of milk as the nutritional substrate for the bacteria.

To also treat animals that may be infected without showing signs of mastitis would require a blanket treatment of all heifers, which is currently neither supported by labels nor good clinical practice.

The majority of studies have demonstrated that treatments are most successful when performed between four and one week before calving. The following treatment options exist:

- Dry cow products require only one tube and, depending on the product, provide a lasting effect. However, the intramammary route of application bears a strong risk of getting kicked by an animal unfamiliar with the procedure. Also, the ceratine plug acting as a natural physical barrier against environmental bacteria needs to be removed for treatment. Finally, the residue situation is not always clear for a dry cow product applied relatively shortly before calving.
- Intramammary tubes for lactation therapy are also an option. The question, however, is whether a one time application is sufficient to remove the infection, while a more than once application per quarter according to the label increases labour and handling risk.
- The use of a systemic treatment antibiotic such as penethamate eliminates the handling risk while allowing for a labelled application regimen that provides effective treatment.



Managing Mastitis 9

Visual check of the milking machine

The milking machine is probably the most important equipment on a dairy farm.

Because it may play a major role in the likelihood of mastitis, like any other mechanical piece of equipment, the milking machine requires regular servicing and maintenance.

However, there are a few simple checks that can be carried out routinely to ensure a good state of the different parts of the milking machine.

These checks do not require specific skills as they are based on visual observations. Done periodically in a preventive way they may help to pinpoint possible problem areas.

It is advised to do these visual checks on a weekly basis and to record observations and measurements.

● **The vacuum pump.**

The vacuum pump is the heart of the milking machine. Its purpose is to remove air from the system, thus creating a vacuum. Vacuum pumps may be divided into either oil or water pumps.

In the case of oil pumps, the belts are checked (breakage, wear, tension, presence of oil) and exhaust controlled; motor and filters should be kept clean and oil changed regularly.

In the case of water pumps, hoses should be checked and replaced at the first sign of leakage. They may get clogged with algae or scum. The water temperature should be accurate (40°C).

The water must not contain chalk residues nor algae. Check the balance tank; the drain on the bottom should seal off. If old and rusty, replace it.

● **Vacuum controller (regulator).**

This holds the vacuum level constant by removing atmospheric air from the system. Check the air filtration system, the outer and inner cleanliness and the diaphragms.

Pulsator.

The pulsator is the device that alternates vacuum and atmospheric air in the pulsation chamber (space between the liner and the teat cup shell). Pulsation is respon-

sible for the milking process. Check the cleanliness, air filtration system and rubber parts. Pulsation may actually be evaluated by examining the teat end of every cow after milking: congestion, oedema or teat end damages indicate malfunctioning.

● **The milking cluster.**

The cluster includes the liner, shells, and claw. Liners are the most crucial parts as they are the only piece of the milking machine that come into direct contact with the cow. They only last for a limited period and must be replaced at prescribed intervals.

Check the condition of the inner surface of the liner, internal and external cleanliness of the claws and collecting tubes. Verify that these parts are not broken, in bad state, deformed or damaged. The status of the rubber connection tubes needs to be checked as well.

● **Line vacuum.**

The vacuum level at which cows are milked has a dramatic effect on the milking operation. Accurate vacuum level may be measured with a vacuum gauge or a mercury manometer. Both devices can give distorted readings. Gauges get wet and/or dirty and lose accuracy. Unusual fluctuations of vacuum level indicates that the controller is dirty and sticking.

● **Milk lines.**

Check the inner cleanliness and the status of filters. They should retain no dirt nor residues of water or milk. In case of systems controlled by valves, their movements as well as the diaphragms need to be assessed. Check the status of all rubber parts and backflusher when they exist.

In order to assess the efficiency of routine visual checking, it is advised to record the results and verify the impact of actions implemented. When no improvement is observed, a thorough detailed checking of the possible causes of flaws must be performed and problems fixed by the milking machine dealer or manufacturer before the next subsequent visual check is executed.



Testing penicillin resistance of S. aureus

With variations between countries, major pathogens such as streptococci, staphylococci and *E. coli* are responsible for the majority of mastitis cases, with *Staphylococcus aureus* being the most frequently isolated species.

Staphylococcus aureus may cause subclinical and clinical mastitis and induce persistently high SCC in dairy cows.

Elimination of this organism from herds requires a combination of antibiotic treatment of infected quarters and aggressive culling of recurring animals.

Antibiotic therapy, especially when performed during lactation, may sometimes yield disappointing results because *S. aureus* chronic infections often show an intermittent shedding pattern due to the deep localisation of *S. aureus* in scar tissues and microabscesses.

However, the main factor for reduced effectiveness of antibiotic treatment still must be seen in the antimicrobial resistance of *S. aureus*.

Beta-lactam antimicrobial agents, such as the penicillins and cephalosporins, are commonly used to treat mastitis and have proven to be highly effective.

Beta-lactamase production is the common mechanism of resistance in *S. aureus* strains.

Beta-lactamases are enzymes that render bacteria resistant to beta-lactam antibiotics. A wide correlation is reported between beta-lactamase production of bacteria and resistance to penicillin G.

When β -lactam antibiotics are used for treatment, the cure rate of penicillin sensitive *S. aureus* is higher than that of beta-lactamase producing strains; the probability of cure is still lower for β -lactamase producing, penicillin resistant *S. aureus* than for

penicillin sensitive *S. aureus* when non- β -lactam antibiotics are used.

Therefore, to measure the beta-lactamase production rate of bacteria is a valuable method to determine the potential penicillin resistance and to evaluate chances of cure.

Bacterial isolates may be easily tested for beta-lactamase production using the nitrocefin containing disk method, by which colony material from a 24 hour culture of a *S. aureus* strain isolated from an infected quarter is applied to the surface of a nitrocefin disk and observed within 15 to 60 minutes. A change in the colour indicates beta-lactamase production.

Studies have shown that MIC (minimum inhibitory concentration) values were consistent with results of the disk diffusion tests.

Average MIC₉₀ (value at which 90% of bacteria are at or below MIC) values for penicillin G are found lower for beta-lactamase negative isolates compared to those for beta-lactamase positive isolates. It was concluded that beta-lactamase production was a good indicator of penicillin resistance.

In addition, it was observed that the results of the nitrocefin test are homogeneous within a dairy herd allowing to draw the penicillin sensitivity pattern of the herd based on three isolates of *S. aureus* from two different cows.

Considering that penicillin resistant strains are far less likely to respond to available treatments than penicillin sensitive strains, one could consider all penicillin resistant *S. aureus* infections ineligible for treatment and, therefore, limit antimicrobial susceptibility testing of *S. aureus* to testing for β -lactamase production.

Mastitis is a local inflammatory process

Mastitis is an inflammatory condition of the mammary gland characterised by signs such as heat, redness, pain and swelling.

Responding to injury, whether by trauma or by infection, the release of inflammatory mediators and modulators induces changes in local microcirculation such as arterioles dilation and increased blood flow.

Meanwhile, pain results from sensitisation of receptors to the actions of locally released chemicals. In a cow udder, this will translate into local signs which may include elevated temperature, swelling of the quarter(s), pain, milk leakage and the modification of milk appearance (presence of flakes, clots, watery aspect). It is quite common to observe a slight or marked swelling of the infected quarter(s) and to notice a restless cow, kicking her own udder, probably owing to irritation and pain.

While these symptoms are quite obvious in cases of acute mastitis, less severe cases of mastitis originate from the same underlying condition of an inflammation. While general symptoms are not apparent, the mammary gland as the origin of trauma or infection still suffers from the same symptoms, less obvious and visible, but most likely to the same detriment. For instance, a cow suffering from moderate mastitis usually does not act as if under pain, but the presence of pain may sometimes indirectly be concluded from a modified behaviour or an altered milk release pattern.

Anti-inflammatory drugs are applied via injection for all inflammatory indications including that of acute mastitis in cows. Their use is best understood in cases where bacterial endotoxin is released and/or when general sign of the disease such as gen-

eral depression, fever, increased heart rate or inhibition of rumen motility are present. They are, however, also helpful in less severe cases of mastitis because mastitis by definition is an inflammatory condition of the mammary gland.

Corticosteroids such as prednisolone have been used widely in the treatment of clinical mastitis. Their usefulness as a local and concomitant treatment of mastitis via incorporation in antibiotic tubes is not always clear. Questions about the relevant dosing in an intramammary combination and the rationale of a dosing regimen that rather follows the need for antibiotic therapy than that of an inflammation control remain unanswered.

It has been suggested that the principal value may be seen in a suppression of the irritation which accompanies intramammary infusions rather than any direct impact on the disease.

Modern NSAIDs have consistently proven their effectiveness in controlling signs of inflammation. They show, however, differences in their pharmacological properties resulting in different administration regimens (single or multiple shots required to achieve full control of inflammatory symptoms) or variable efficacy on local signs. Indeed, as availability in the udder tissue is not identical between drugs, only few are able to reach the udder and achieve effective concentrations to alleviate and control local signs in acute and also in less acute cases.

While given via injection, clearly, those products will be most suitable for therapy which express their activity in controlling inflammatory signs also at local level making this an important criterion for selecting the right product. ■

Extended antibiotic therapy for mastitis

The key objective of treating cows suffering from clinical or subclinical mastitis with an antibiotic during lactation is to cure the infected quarter. However, if the assessment of cure is only based on a return to normal aspect of the milk and udder, or on a transient decrease of somatic cell count, in many cases cure will only be partial or temporary with a high risk of relapses. Therefore, ideally, the treatment with antibiotics of an infected quarter must lead to bacteriological cure, or, essentially, to the elimination of the pathogen from the udder. However, even when a clinical cure is achieved, bacteriological elimination rarely exceeds a rate of 60%.

This may explain the increasing trend amongst commercial dairy farmers and veterinary practitioners – particularly on recurrent cases – to increase the amount of drug administered per application, or to extend the total duration of treatment, both options infringing on the label recommendations. Such off label use of drugs may include extended treatment periods with either parenteral or intramammary antibiotic treatments or the combination of both. In some cases, it may be an aggressive intramammary antibiotic treatment at every milking over three to six days.

Though all these strategies aim at achieving a satisfactory cure of disease and the restoration of milk quality, the scientific justification for such practices is not always given or at least obvious.

Extending the duration of treatment is, for example, useless for antibiotics for which the efficacy is concentration dependant (the higher the dose, the better the efficacy). On the other hand, an increased killing rate is not to be expected if the pharmacological profile of an antibiotic does not allow an appropriate concentration at the site of infection to be achieved. This will not be changed by an increased duration of treat-

ment. Last but not least, studies have shown that the combination of parenteral and intramammary therapy does not always provide a significantly higher cure rate than either of the therapies alone.

However, extended therapy regimens may enhance the cure rate achieved by intramammary antibiotic treatment. Most of the approved protocols for intramammary antibiotics recommend the use of one to three syringes per case, either once daily or after every milking. Some protocols might be ineffective or inappropriate with regard to the goal of cure because regimens are only based on a quick milk return to sale or short milk withdrawal time rather than on quality standards (such as bacterial count or somatic cell count).

By increasing the frequency of administration or the duration of the treatment, a quicker and higher cure rate can be expected. However, the latter is not always justified by the economic benefits of such treatment and the risk of new contaminations of the teats may increase with more frequent intramammary applications.

Recent publications have shown that the additional benefits from an extended duration of treatment with parenteral antibiotic are often less obvious. Clinical or bacteriological cure rates are only marginally improved and – unless the duration is extended by four times – rarely worth the additional costs. This may be explained by the fact that parenteral treatment with an antibiotic able to reach the udder leads to local antibiotic concentrations, even in the deepest udder tissue, which are sustained and effective. Further, this concentration is not affected by regular milking.

In general, extended treatment with antibiotic results in longer lasting positive inhibitory tests for milk. This implies increased volume of discarded milk and increased overall treatment costs. ■