Milking hygiene: new issues and opportunities from automatic milking

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Paper received June 26, 2003; accepted August 28, 2003

ABSTRACT

Automatic milking offers the opportunity of in-line measurements of milk components, check of milking and cleaning procedures, and surveillance through the management program. These advantages may directly benefit the milk quality. Diversion of abnormal milk at time of milking is critical to the milk quality. It is proposed to define abnormal milk as milk being visibly changed in homogeneity or colour from that of normal milk. Several enzymes and other milk components may be involved in the formation of clots in the milk. Based on reported changes in primarily the milk protein fraction as a response to infection of the mammary gland, different explanations for the generation of precipitates in mastitis milk are discussed. Automatic milking systems (AMS) should have sensors to monitor and divert abnormal milk. The management system of AMS provides an excellent opportunity to introduce Hazard Analyses Critical Control Points (HACCP) systems for surveying the milk quality. HACCP-based proposals are given for avoiding contamination of the bulk milk with milk from cows with clinical mastitis and for ensuring a low bulk milk bacterial count.

Key words: Dairy cows, Automatic milking, Mastitis, Hygiene, HACCP

Introduction

Automatic milking offers the opportunity to standardise the milking procedures to a very large degree, to make individual settings for individual cows or group of cows, and to take benefit from in-line measurements of milk components. The potentials and perspectives of improving the milk quality by milking automatically are obvious but goals have to be focussed and well defined. Introduction of automatic milking has resulted in degradation of the milk quality in Denmark reflected in a higher bulk milk cell count, higher total bacterial count, higher count of spores of anaerobes, and a higher freezing point of the bulk milk (Rasmussen et al., 2002). Likewise, Klungel et al. (2000) found an increase in total bacterial count and freezing point of milk from Dutch farms. Rasmussen et al. (2002) concluded that the increase in bulk milk somatic cell count indicated that milk from clinically infected cows and cows with high cell counts were not diverted to the same degree when milking automatically as when milking conventionally. The increase in total bac-
At present six companies in Europe produce several models of automatic milking systems (AMS) and they do (with one exception) not sort milk according to pre-set requirements. One model measures the colour of the milk and may divert reddish milk as a result of contamination with blood. So far international thresholds for discarding milk of inferior quality have not been set. However, the International Organisation for Standardisation has established a group to form general standards for AMS. Additionally, one work package of the EU-project "Implications of the introduction of automatic milking on dairy farms" (see www.automaticmilking.nl) aims at giving a definition of normal and abnormal milk. The objectives of the present paper are to discuss the implications for the milk quality of these definitions and to give examples of hazard analysis of critical control points (HACCP) that may improve and stabilise the milk quality.

**Definition of normal and abnormal milk at time of milking**

The general conditions of hygiene in milk production in the EU are defined by the Commission Directive 89/362/EEC (1989). Within the EU-project, a workshop was held in November 2002 on the definition of normal and abnormal milk at time of milking. As a summary of the workshop, Rasmussen (2003) proposed to replace the current part of the hygiene directive with the text:

*Milking must be carried out hygienically ensuring in particular: - that milk from an animal is checked for abnormalities by the milker or a method achieving similar results and that only normal milk is used for human consumption and that abnormal, contaminated, and undesirable milk is excluded.*

This text is based on the following definitions of normal, abnormal, contaminated, and undesirable milk:

- **Normal milk:** Milk suitable for human consumption.
- **Abnormal milk:** Milk which differs from normal milk in respect of colour or homogeneity.
- **Contaminated milk:** Milk which, prior to the milking of the animal, is known to be unfit for human consumption following treatment of the animal with antibiotics or other veterinary products, which have a requirement that the milk must be withheld from sale for such use.
- **Undesirable milk:** Milk which, prior to the milking of the animal, is known to be unsuitable for human consumption, e.g., colostrum and milk with high somatic cell count.

This definition requires that the AMS monitors the milk quality at the time of milking in order to exclude milk from cows with clinical mastitis. The frequency of visible blood in the milk is rare but also regarded as abnormal. The conditions "contaminated milk" and "undesirable milk" are (or should be) known prior to milking and do as such not require additional monitoring. Secretion from the udder in the first 3 days after calving is mainly colostrum, which is not regarded as "normal" milk. A high somatic cell count (SCC) indicates inflammation in the udder and may be possible to monitor automatically. However, since the definition has to apply to conventional milking as well, SCC cannot be required to be measured at every milking for determination of abnormal milk.

**Abnormal milk and milk composition**

According to the current hygiene directive and the proposal, milk from cows with clots in the milk must be withheld and the question arises what effect this has on the milk quality. Schmidt (1971) stated that "casein in the milk is precipitated in some instances, but the coagulation of the blood fluids accounts for most of the flakes and clots in the milk". Based on observations from filtration experiments (100 µm pores) of mastitis milk, it truly appears that in some mastitis milk samples red blood clots are present, but filtration of other samples results in the presence of light coloured unidentified precipitated material in the retained fraction (Rasmussen, 2003).

The change in composition of milk from cows and quarters with clinical mastitis is characterised by a number of changes in enzymes, min-
erals and trace elements, whey proteins, caseins, and the major milk components (Kitchen, 1981; Korhonen and Kaartinen, 1995). SCC increases as a result of influx of especially neutrophils into the milk in response to a variety of inflammatory mediators (Nickerson and Pankey, 1984, Sordillo et al., 1997). More than 90% of the leucocyte population was found to consist of neutrophils within 6 hours after a challenge to bacterial endotoxin (Saad and Östensson, 1990), and the relative distribution of cells in milk from cows with prolonged subclinical mastitis was found to be 52% neutrophils, 39% macrophages, and 9% lymphocytes (Azzara and Dimick 1985).

The composition of milk approaches that of blood during clinical mastitis. There is a low level of total solids in mastitis milk, especially due to a lower level of lactose. This is accompanied by an increase in sodium and chloride from blood to compensate osmotically for the lower lactose synthesis, and the pH increases to approach that of blood (Fox and McSweeney, 1998). The total protein concentration of the milk does not change much, but the ratio between caseins and whey proteins is changed. This is explained by a depression of proteins largely synthesized by the mammary gland (caseins, α-lactalbumin and β-lactoglobulin), while the levels of proteins and enzymes derived from blood increase, including e.g. immunoglobulins, bovine serum albumin, catalase and NAGase (Kitchen, 1981). Also, the proteolytic potential of the milk is increased (Andrews, 1983, de Rham and Andrews, 1982; Verdi et al, 1987). The increase in proteolytic activity may come from an increase in bovine enzymes, proenzymes and/or activators coming from either milk leucocytes, from the pathogen itself, secreted by the mammary epithelium or may be transferred to milk from blood, like plasminogen (Politia et al, 1989).

Neutrophils aid in the immunological defence mechanism of the udder and contain very active acid and neutral lysosomal proteases, like elastase and cathepsins B, D and G (Kirschke and Barrett, 1987; Travis, 1988), which can cleave milk proteins when secreted or released from the cells.

What makes the milk clot?

Indeed, there may be more than one explana-
tion for the occurrence of light coloured flakes and clots in milk from infected glands, and probably the effects act together or are more or less pronounced in different types of mastitis milk. Different explanations of these observations, which are only scarcely covered by literature, are discussed below, but it must be emphasised that other explanations may just as well be true.

Agglutination

The level of immunoglobulins in the milk increases dramatically during clinical mastitis. It seems likely that some of the aggregates in mastitis milk consist of immunoglobulins and eventually other whey proteins agglutinated with fat globules, as creaming of a cow's milk is enhanced by addition of blood serum or colostrum to the milk; of which especially immunoglobulin M is responsible for the reaction (Euber and Brunner, 1984; Fox and McSweeney, 1998). As the aggregation occurs at temperatures below 37°C, these immunoglobulins have been called cryoglobulins. Apart from temperature, the aggregation is influenced by ionic strength and pH. The cryoglobulins may occur as precipitates on the surface of fat globules, causing them to agglutinate or they may form a network in which the fat globules are entrapped (Fox and McSweeney, 1998). Eventually, also bacterial agglutination by immunoglobulin A may occur (Sordillo et al., 1997).

Changes in casein fraction

The concentrations of αs1- and (κ-casein were found to be reduced in milk after infusion with bacterial endotoxin, and inversely related to somatic cell count (Anderson and Andrews, 1977). Glycosylation of the C-terminal part of κ-casein has been found to stabilise the casein micelle (Dziuba and Minkiewicz, 1996), and therefore changes in the glycosylation of κ-casein induced by inflammation may destabilise the casein micelles.

Mastitis causes an increase in the ratio of soluble to micellar casein, where in normal milk the micellar casein may represent up to 95% of the total casein, while the level of micellar milk in milk from infected animals may be as low as 46% of the total casein (Kitchen, 1981). However, it is not clear whether these changes in the casein frac-
tion are able to induce direct precipitation to occur in mastitic milk.

**Proteolytic activity**

Para-κ-casein was shown to be present in high cell count milk after infusion with bacterial endotoxin, while no para-κ-casein was present in pre-infusion milk (Anderson and Andrews, 1977). A positive correlation between somatic cell count and para-κ-casein was also found in another study (Rogers et al., 1989). The para-κ-casein fragment is generated from intact κ-casein by proteolytic cleavage of the Phe104-Met105 bond, e.g. by chymosin or other proteases, resulting in liberation of the C-terminal glycomacropeptide fragment, and precipitation of casein micelles, due to destabilisation after liberation of this hydrophilic polypeptide. The para-κ-casein content was found to reach 25% of the initial κ-casein content in infection studies (Anderson and Andrews, 1977). It therefore seems likely that proteases present in mastitis milk or high cell count milk are responsible for this proteolytic cleavage of κ-casein. One of the proteases of milk, cathepsin D, has in fact been shown to be able to cleave the Phe104-Met105 peptide bond in κ-casein, and furthermore to be able to coagulate milk when added to normal milk (McSweeney and Fox, 1995; Larsen et al., 1996). The cathepsin D activity in milk was found to positively correlate with cell count (O-Driscoll et al., 1999). But it is not clear whether the potential level of cathepsin D, or other somatic cell related proteases, would be sufficient to induce generation of para-κ-casein in an amount leading to precipitation of caseins in mastitis milk.

Investigations are currently being carried out in our laboratories to study these different explanations for the generation of flakes and clots in different types of mastitis milk, with the aim to find an objective marker for clotted milk.

**Consequences of withholding abnormal milk for the bulk milk SCC**

Degradation of the processing properties and product quantity and quality will be the consequence of including mastitis milk in the bulk milk. The enzyme activity is highly correlated with the SCC and probably especially to the neutrophils. Exclusion of visually abnormal milk will lower the SCC but will not ensure that all quarters have low SCC, see Table 1. If the goal of a milk production based on automatic milking is to achieve very low bulk milk SCC, the sorting tool should be based directly on this property. There seems to be a milk quality benefit of sorting milk based on proteolytic enzymes but rapid and automatic detection methods have not been developed so far. Probably, the most important aspect of diverting milk from cows with clinical mastitis is the image and aesthetic aspect of milk production. It is up to national or regional regulations to define the acceptable level of undesirable milk in bulk milk.

**Application of HACCP principles to automatic milking**

Many food-processing factories apply HACCP to ensure the quality of their products. EU has discussed this subject for the primary production as well but decided not to require this in the coming hygiene directive. However, in-line measurements and good management routines of AMS offer the opportunity to control the production of raw milk to a standard that exceeds conventional milking. The general principles of HACCP consist of 7 steps (Cullor 1997): 1: Identification of potential hazards; 2: Description of procedures or processes where the hazard can be controlled (critical control point = CCP); 3: Set thresholds for each CCP; 4: Set time schedules and measurements for each CCP; 5: Describe actions in case that CCP is not under control; 6: Set up verification systems to prove that HACCP works; 7: Document all steps and actions.

**Control for clinical mastitis**

In the following, an example is given of avoiding the mixture of the bulk milk with milk from cows with clinical mastitis when milking automatically. Numbers relate to the 7 steps of HACCP.

1. Mastitis is a multifactorial disease and many risk factors exist. Thus potential hazards are many and not all will be discussed in details, but only very general preventive actions will be considered here. We expect the risk to increase with increasing prevalence of clinical mastitis. Consequently, all incoming cows of...
the herd should be free of mastitis, cows should be kept dry, clean, and comfortable, the milking machine should perform to international standards, and the immune system of the cows should be intact.

2. Inspect the milk and palpate udders of all newly calved cows and of purchased animals and repeat this every week for three weeks. Take quarter milk samples of these cows to check for subclinical mastitis. Inspect the filter of the bulk tank. Score the dirtiness of cows. Inspect the warning list for malfunctioning of the milking machine. Inspect the general health of all cows.

3. A maximum of 5% of the incoming cows may have clinical mastitis. Less than 20% of the animals should test positive for subclinical mastitis. No clots from clinical mastitis may be visible at the bulk milk filter and less than 5% of the filter surface should be covered with dirt or manure. Less than 5% of the cows should have dirty legs and all udders should be clean. All stalls should be dry. The milking machine should fulfill ISO-standards. All cows should be healthy.

4. Register the daily clinical observations and milk inspections in the management program of AMS where reports are developed to survey thresholds. Inspect the milk filter at every change (= 3x daily). Inspect all stalls and all cows once daily and file observations above the threshold in the management program. The management program will automatically come up with a warning if the function of the milking machine fails.

5. Treat or cull all cases of clinical mastitis immediately. Discuss rearing and dry cow facilities and methods with a consultant. Replace suppliers. Add an additional cleaning of stalls and alleys daily and change bedding more often. Call a technician to correct milking machine failures. Call the veterinarian for the treatment of cows, discuss prophylactic procedures specific for the observed disease, and take action.

6. Verification of the HACCP system is done by the dairy factory’s monitoring of bulk milk SCC at every delivery and by letting the veterinarian check the database of the management program. Documentation is entirely kept in the management program of the AMS where all actions are filed.

7. Control for high bulk milk bacterial count
   The following is an example of a program to control the total bacterial count of the bulk milk not to exceed the limit for premium class.

   1. Bacteria in the bulk milk originate from contamination of the teat surface, clinical mastitis, insufficient cleaning of the milking equipment, insufficient cooling, or long storage time of the milk.

   2. Inspect for clinical mastitis and clean udders as above (no further reference is given to these two points since they have already been discussed). Check the cleaning frequency, cleaning temperature, pH of the cleaning and disinfection solution. Inspect the flow of the washing solution at critical points of the milking equipment and of the bulk tank.

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**Table 1. SCC of quarter milk and percentage of quarters > 200,000, > 400,000 and >1*10^6 cells/ml by visual appearance of foremilk (Rasmussen, 2003).**

| Appearance of the foremilk | n. | Logarithmic mean | Arithmetic mean / ml | % of samples |
|----------------------------|----|------------------|----------------------|--------------|
|                            |    |                  |                      | >200,000/ml  | >400,000/ml | >1*10^6/ml |
| Normal                     | 1362| 4.8              | 295,000              | 15           | 9           | 4          |
| Watery and flakes          | 22  | 6.1              | 3,573,000            | 86           | 77          | 59         |
| Clots in the milk          | 40  | 6.8              | 12,269,000           | 93           | 93          | 88         |
Measure the temperature in the milk delivery lines. Monitor the storage temperature of the bulk milk.

3. The milking equipment should be cleaned at least every 10 hours and the bulk tank after every delivery to the dairy factory. Pre-rinsing temperature should be between 30-40°C, the end-temperature of the washing solution >40°C, pH of the alkali cleaning solution >11 and <2 of an acid disinfection solution. The surface of the critical cleaning points should be covered with the cleaning solution for >1 min. The temperature of the milk delivery lines should be <20°C and milk stay there for <30 min. Maximum storage temperature should be <8°C and mean storage temperature <4°C.

4. The AMS is equipped with the specific sensors for monitoring of temperature, pH (or conductivity), and flow at the control points. A self-check of the sensors is built into the system. The management program surveys the measurements with respect to the pre-set thresholds and gives an alarm in case of failures.

5. The management program proposes the most likely causes for the observed failures. Corrections are made immediately or a technician called to fix the problem.

6. The dairy factory surveys the bulk milk total bacterial count of milk samples from every delivery and early warnings are given by a built-in surveillance program analysing trends of the bacterial counts.

7. All measurements are stored and all actions filed in the AMS management program.

Discussion

High bacterial and cell counts or clots in the milk are not hazards to the human health as long as pasteurisation of the raw milk at the dairy factory is functioning. The benefits of the proposed HACCP programs are mainly monitoring of udder health and milk quality in order to keep premium prices, ensuring a high income for the farmer, and providing the opportunity for fast action in case of failures and before milk quality deductions are endangered. The two examples above are presented in general terms but specific programs have to be adapted to the conditions at the individual farms. A critical point in diverting milk from cows with clinical mastitis is the sensor for detection of clots in the milk. Proposals have been given of milk components that may be involved in clotting of the milk but indirect methods may apply as well. Development of such sensors is a huge challenge for the AMS manufacturers but necessary in order to keep the image of a healthy milk production from cows milked automatically. Development of in-line sensors monitoring proteolytic activity may further improve the milk quality for processing.

Conclusions

Diversion of abnormal milk at time of milking is critical to the milk quality. Abnormal milk is defined as milk visibly changed in homogeneity or colour from that of normal milk. Several enzymes and other milk components may be involved in the formation of clots in the milk. AMS should have sensors to monitor and divert abnormal milk. The management system of AMS is an excellent opportunity to introduce HACCP systems for surveying the milk quality.

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