Udder health of dairy cows in automatic milking

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Veterinary Medicine of the University of Helsinki, for public examination in Walter Hall, Agnes Sjöbergin katu 2, Helsinki, on 16th October 2009, at 12 noon.

Helsinki 2009
To my family
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ABSTRACT

Automatic milking (AM) is increasing in modern dairy farming, and currently over 5000 farms worldwide use this technology. The automatic milking system (AMS) is designed to replace conventional milking (CM), managed by a milker in a milking parlour or in tie stalls. In AM, cows are generally milked more frequently than in CM, and milking is quarter-based instead of udder-based. Despite improvements to the milking process, udder health has not improved in AM. This thesis focuses on udder health of dairy cows in AM. The effect of a change from CM to AM on udder health was studied. Additional aims of the work were to investigate different methods of automatic mastitis detection and teat cleaning to document possible risk factors for mastitis in AM. Finally, good management methods in AM are suggested based on the results of the work that represents this thesis.

Udder health of dairy cows of 88 herds that were changed from CM to AM was investigated and compared with udder health of cows of 94 herds that were changed from tie stall barn to free stall barn with CM. Milk record data from the first year before and after the change were compared. Calculations were made for logarithmic cow somatic cell count (logSCC), proportion of cows at risk with SCC > 200 000 cells/ml for the first time (highSCC) and number of treatments of clinical mastitis within a herd. Udder health of cows that were changed from CM to AM deteriorated slightly more during the change compared with the group for which only the type of the barn was changed; logSCC increased from 4.89 to 4.96 and the proportion of highSCC cows from 2.8% to 3.8%, compared with an increase in logSCC from 4.87 to 4.90 and in the proportion of highSCC cows from 2.0% to 2.2%. In both groups, logSCC increased already before the change, and an apparent adaptation period of 2 to 3 months was recorded after the change from CM to AM. The proportion of highSCC cows appeared to stabilise near to the original figures towards the end of the study period in both groups, but no improvement was noted in logSCC in the herds changed from CM to AM. The number of recorded treatments of cows that were changed from CM to AM decreased during the change from 5.3 to 5.1 treatments per 10 000 cow-days, compared with an increase from 3.0 to 4.6 treatments per 10 000 cow-days for herds for which only the barn was changed.

Technical success and effectiveness of teat cleaning and management factors associated with them were evaluated in nine AM herds. On average, 80% of 616 teat cleanings with a cleaning cup, and 85% of 716 teat cleanings with rotating brushes were technically successful, i.e. the teat was correctly positioned in the cleaning device throughout the whole cleaning process. Technical success of teat cleaning was strongly dependent on herd characteristics. Other factors associated with the technical success of teat cleaning with a cleaning cup were days in milk, behaviour of the cow, teat colour, and teat position (fore or hind). Excessive udder hair or technical failure of the AMS caused a few technically unsuccessful teat cleanings in the group where a cleaning cup was used. For rotating brushes, behaviour of the cow, teat position, udder and teat structure, and days in milk was associated with technical success.

Teats for which technically successful teat cleanings were made were evaluated for effectiveness of teat cleaning. For originally dirty teats the cleaning cup was significantly more efficient than the brushes, with a higher percentage of teats becoming clean or
almost clean during the cleaning process (80% vs. 73%). Teat orifices were less efficiently cleaned compared with teat barrels and teat apices. Bedding material (peat, sawdust, or straw) adhering to the teat was almost entirely removed. Factors associated with the effectiveness of teat cleaning were teat cleanliness before cleaning, herd, teat cleaning method and teat end condition.

Efficiency and precision of mastitis detection methods in AM were evaluated in the same herds as teat cleaning, using eight herds divided into two groups of 4 + 4 herds (Groups A and B), based on the AMS brand used. For subclinical mastitis data, herds were visited once to collect milk samples. In addition, the farmers followed up alerts generated by the AMS for 15 to 30 days, to study the reliability of the alerts. They also recorded all cases of clinical mastitis detected using any means during six months. Sensitivity of detecting quarters with an SCC over the threshold value of 200 000 or 1 000 000 cells/ml was 18% to 43% based on electrical conductivity (EC) in Group A and 6% to 13% based on EC and milk colour in Group B. Specificity was > 97.0%, although in Group A, 6/17 of the EC alerts were false positives. All seven EC alerts in Group B were true positive.

On the day on which the farmer detected clinical mastitis, 4/7 and 14/17 quarters were associated with alerts in Groups A and B. During the follow-up period, 58% (Group A) and 27% (Group B) of the cows were associated with alerts based on EC. The number of alerts was halved in Group A when a running average was applied to the criteria of EC alerts. The proportion of true positives simultaneously increased from 30% to 60%. In Group B, the number of alerts was almost doubled with alert criteria manipulation, but the proportion of true positives declined, as expected, from 80% to 70%. In addition, during the follow-up period, 84% of the cows were associated with alerts based on deviations in milk yield in Group A and 12% based on milk colour in Group B. Of the alerts based on milk yield or milk colour 20% and 60% to 90% were true positives.

To test a new method for detecting clinical mastitis, use of a thermal camera image was studied. Mastitis was experimentally induced in 6 cows with 10 µg of Escherichia coli lipopolysaccharide infused into the left fore quarter of each udder, and the right fore quarter served as a control. The first systemic and local signs of clinical mastitis were noted in all cows 2 h after the induction. Rectal temperature, milk SCC, and EC increased 4 h after the induction. The thermal camera successfully detected >1 °C temperature change on the udder skin of all cows, parallel with the rise in rectal temperature, but local signs on the udder were recorded before the rise in the temperature of the udder skin. A thermal camera mounted in a milking or feeding parlour in AM could be useful for detecting temperature changes associated with clinical mastitis and other febrile diseases, and the method is non-invasive and is not related to milking.

The results of this thesis suggest that udder health has slightly deteriorated during the first year after introduction of AM, and the effect is more pronounced than if only the type of the barn is changed. Automatic detection of subclinical and clinical mastitis and cleaning the teats before milking represent challenges. Failures in mastitis detection and milking hygiene pose a risk for udder health. These risk factors can partly be controlled by management actions taken by the farmer, but there is still a need for further technical development of AM. In order to maintain good udder health in AM, it is imperative that the barn is properly designed to keep the cows clean, that cow traffic is fluid, and that the
AM machine works effectively. Careful observation of the cows and knowledge of how to use all data gathered from the system are also important. Despite all the many advances in milking technology and herd management, there remains an overriding need for experience and observation on farms employing AMS. “Automatic” does not indicate that the role of the herdsman is in any way diminished.
This thesis is based on the following publications:


The publications are referred to in the text by their roman numerals. The original articles are reprinted with the kind permission of their copyright holders: American Dairy Society (I, II and IV), and Taylor & Francis (III).
# ABBREVIATIONS

<table>
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AM</td>
<td>automatic milking</td>
</tr>
<tr>
<td>AMU</td>
<td>automatic milking unit</td>
</tr>
<tr>
<td>AMS</td>
<td>automatic milking system</td>
</tr>
<tr>
<td>BMSCC</td>
<td>bulk milk somatic cell count</td>
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<tr>
<td>CNS</td>
<td>coagulase-negative staphylococci</td>
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<td>CM</td>
<td>conventional milking</td>
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<td>CMT</td>
<td>California Mastitis Test</td>
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<td>DIM</td>
<td>days in milk</td>
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<td>EC</td>
<td>electrical conductivity</td>
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<td>ETC</td>
<td>effectiveness of teat cleaning</td>
</tr>
<tr>
<td>FP</td>
<td>false positive</td>
</tr>
<tr>
<td>IMI</td>
<td>intra-mammary infection</td>
</tr>
<tr>
<td>IRT</td>
<td>infra-red thermography</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
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<td>MI</td>
<td>milking interval</td>
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<tr>
<td>NAGase</td>
<td>N-acetyl-β-D-glucosaminidase</td>
</tr>
<tr>
<td>NPV</td>
<td>negative predictive value</td>
</tr>
<tr>
<td>PPV</td>
<td>positive predictive value</td>
</tr>
<tr>
<td>SCC</td>
<td>somatic cell count</td>
</tr>
<tr>
<td>SE</td>
<td>sensitivity</td>
</tr>
<tr>
<td>SP</td>
<td>specificity</td>
</tr>
<tr>
<td>TP</td>
<td>true positive</td>
</tr>
<tr>
<td>TR</td>
<td>rectal temperature</td>
</tr>
<tr>
<td>TSTC</td>
<td>technical success of teat cleaning</td>
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<td>TU</td>
<td>udder skin temperature</td>
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Automatic milking (AM) is one step in series of measures taken to automate dairy production. In the Nordic countries dairy cows have traditionally been kept in tie stall barns and herds have been small. The dairy industry is currently undergoing structural changes because small farms are no longer profitable. Farms are becoming larger and because of high labour costs automation is becoming more common (Table 1), as over 5000 AM farms exist worldwide.

Table 1. Number of farms with an automatic milking system (AMS) and number of automatic milking units (AMU) in the Nordic countries at the end of 2008 (Mats Gyllenswärd, Svensk Mjölk, representing NMSM (Nordic Dairy Association's Committee for Milk Quality), personal communication 2009).

<table>
<thead>
<tr>
<th></th>
<th>Finland</th>
<th>Sweden</th>
<th>Denmark</th>
<th>Norway</th>
<th>Iceland</th>
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<tr>
<td>AMS farms</td>
<td>385</td>
<td>553</td>
<td>832</td>
<td>447</td>
<td>97</td>
</tr>
<tr>
<td>AMUs</td>
<td>452</td>
<td>839</td>
<td>1943</td>
<td>460</td>
<td>115</td>
</tr>
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</table>

Concern about cow udder health arises when housing or milking systems change and particularly with increasing herd size. Results on the effects of the introduction of AM indicate deteriorating udder health (Rasmussen et al., 2001; Kruip et al., 2002; Poelarends et al., 2004; Mulder et al., 2004; Rasmussen, 2006). Small-scale studies comparing AM and conventional milking (CM) in similar environments indicate that udder health is more dependent on the quality of overall management of the cows than on the type of milking (Berglund et al., 2002; Hamann and Reinecke, 2002; Shoshani and Chaffer, 2002; Wirtz et al., 2004; Lopez-Benavides et al., 2006).

Udder health may be affected by structural changes in the barn, changes in management and by changes in animal-based factors. Along with the change from CM to AM detection of illnesses, cleanliness, level of exercise, feeding regimes, stress level of the cows and infection pressure change. As farms grow larger, staff time spent per cow decreases and the throughput of cows at milking increases. Cows are bred for higher milk yield, which has been associated with increased incidence of mastitis (Koivula et al., 2005), and for increased milk flow, which has been associated with a higher risk of milk leakage (Persson-Waller et al., 2003). The milking process is different in AM and CM: milking frequency and intervals between milking, milking equipment settings, and procedures for teat dipping differ. Cleaning the teats before milking and detection of mastitis is carried out automatically. Furthermore, AM is quarter-based which prevents the spread of intra-mammary infection (IMI) between teats of a cow, and reduces over-milking. The spread of IMI among cows is no longer managed by milking order, but can be reduced by flushing the liners between individual milkings (Schuiling, 2004).

Mastitis (inflammation of the mammary gland, almost always caused by infecting micro-organism, IDF 1999) decreases milk quality and milk yield, causes economic losses and an increased workload for the farmer, but also can affect animal welfare (freedom from pain, injury and disease, FAWC, 1993). Mastitis is the most frequently treated disease of
dairy cows (Østeräs et al., 2007). To maintain udder health of dairy cows, we need to understand the complex nature of interactions between cow, environment, management and milking. This thesis addresses udder health in AM, with special regard to teat cleaning and mastitis detection.
2 REVIEW OF THE LITERATURE

2.1 Udder health in automatic milking

Epidemiological studies covering large numbers of farms have indicated deteriorating udder health among cows, reflected as high individual cow somatic cell counts (SCC) in milk or acutely elevated cow composite SCC after the introduction of AM (Rasmussen et al., 2001; Kruip et al., 2002; Mulder et al., 2004; Poelarends et al., 2004; Pedersen and Bennedsgaard, 2006; Rasmussen, 2006). In a French study of 46 farms however, no change in cow SCC was recorded (Billon and Tournaire, 2002). Introduction of AM is often accompanied by other changes in the barn; for example, change from a tie stall to a free stall system, an increase in herd size, changes in cow management, and initiation of use of automatically gathered data. To establish the real effect of AM on udder health it is important to minimise the effect of additional alterations made during the change in milking system. Studies on the effect of a change from a tied to a free stall barn on udder health have produced inconsistent results. Hultgren (2002) did not report changes in herd prevalence of high SCC cows, but the frequency of veterinary treated clinical mastitis and teat injuries were lower for cows in free stall barn. Biefeldt et al. (2004) reported a lower mean cow SCC associated with tie stall barns. Increasing herd size has recently been associated with increased proportion of farms having clinical mastitis (Hill et al., 2009), although almost all Finnish farms would be included in the “small” farms in their study. In an earlier study, Bartlett et al. (1992) showed an association between larger herd size and mastitis caused by Streptococcus agalactiae. No association between herd size and the incidence of clinical mastitis was reported in Kossaibati et al. (1988).

Cow composite SCC was higher in AM farms in a comparison of >200 AM and >200 CM farms in Holland (Mulder et al., 2004). Studies comparing AM and CM in similar environments with similar management indicated no differences in udder health of the cows measured as cow SCC (Berglund et al., 2002), mastitis prevalence (Hamann and Reinecke, 2002), or incidence (Wirtz et al., 2004). In other studies udder health, measured as quarter SCC (Berglund et al., 2002), new infection rate and individual cow SCC (Shoshani and Chaffer, 2002), was better in AM. Comparison of two farms operating a pasture-based system revealed a higher incidence of clinical mastitis and prevalence of Str. uberis IMI post partum associated with CM compared with AM, but the CM herd size was twice that of the AM farm and the proportion of heifers was 4 times higher in CM (Lopez-Benavides et al., 2006). Epidemiological studies comparing tie stall and free stall barn indicate better udder health in free stall barns. Matzke et al. (1992) reported a decreased prevalence of cows with high SCC, IMI and teat injuries in free stall barn, and Ekesbo (1966) and Valde et al. (1997) established a lower level of clinical mastitis for cows in free stall barns.

Overall, it seems that udder health can be positively or negatively affected by a change from a tie to a free stall barn, but is negatively affected by a change from CM to AM. Unfortunately, it is not known whether the AM farms studied changed their barn type or herd size at the same time. On the other hand, some small-scale studies comparing milking systems have indicated that udder health is more dependent on the quality of overall
management than on the type of milking. As the results from the studies are controversial, further studies should include parallel comparison of the effects on udder health of different factors and changes in the barn and the management of the cows.

In Finland, national data on bulk milk SCC (BMSCC) for all dairy farms, large farms and AM farms (http://www.maitohygienialiitto.fi/maidon_laatu_autom_03.html) show that the geometrical mean of BMSCC of the AM herds is approximately 27 000 cells/ml higher than that of the respective conventional large farms (>45 cows), and 45 000 cells/ml higher than on all farms, where BMSCC is approximately 121 000 cells/ml.

2.2 Automatic milking process

2.2.1 Number of milkings and length of milking intervals

Milking frequency is increased in AM compared with CM, at least for most of the cows (Hogeveen et al., 2001). In an ongoing study by Neijenhuis et al. (2008), the mean milking frequency on 150 Dutch farms with an average of 62 cows/farm was 2.3/day (range 1.5 - 3.3), which appears low compared with 2.5-3.0/day suggested as a practical experience (de Koning and Rodenburg, 2004). Increased milking frequency flushes bacteria from the udder more often whereas long milking intervals (MI) provide bacteria with time to colonise the teat after bacteria have successfully penetrated the teat canal after milking (Bramley et al., 1981). Frequent milking-out of a quarter has traditionally been used as an empirical mastitis treatment. Dahl et al. (2004) showed lower somatic cell scores for cows milked 6 times/day than 3 times/day in the early lactation. Three times/day milking was shown to result in decreased cow SCC (Klei et al., 1997; Hogeveen et al., 2000; Smith et al., 2002) and a lower number of cows having new increased SCC (Hogeveen et al., 2000) compared with 2 times/day milking. Increased milking frequency resulted in a decreased incidence of mastitis by Str. agalaktiae (Hillerton, 1991). Köhn et al. (2007) established a slightly negative correlation between SCC and milking frequency in 10 farms with AM and free cow traffic. In a study of more than 900 cases of clinical mastitis (Rasmussen et al., 2007), MI increased by approximately 2 hours a day one month before treatment in AM. It was suggested that this indicated that cows’ daily rhythms were affected by the disease and that increased MI possibly also enhanced the development of clinical mastitis, at least in quarters with subclinical infection.

On the other hand, frequent milking provides greater opportunity for bacterial invasion during milking, if milking equipment is not in good working order, and teat canals will be open more often after milking, allowing environmental bacteria to invade the udder (Hillerton, 1991). Short MIs leave less time for the teats to recover from milking, which could have an adverse effect on teat condition (Ipema and Benders, 1992). This can predispose quarters to mastitis (Neijenhuis et al., 2001b). Cow SCC and incidence of new IMIs were not affected by milking 3 times/day as compared with milking twice a day in an experimental study by Waterman et al. (1983). No effect was found on California Mastitis Test (CMT) scores in a study of 28 Californian herds initiating 3 times/day milking (Gisi et al., 1986), but CMT scores were higher for cows milked 3 times/day until the third
lactation, and vice versa in the fourth lactation (Allen et al., 1986). Smolders et al. (2001) had to discontinue their study comparing 2 times/day milking with 7 times/day milking, because of deteriorated udder health in the frequent milking group.

Increased cow SCC in AM was assumed to be mainly caused by the irregularity of milking (Kruip et al. 2002), but there are few data to support this assumption. Stelwagen et al. (2008) demonstrated increased cow SCC (although recurrent) still existing 24 h after a single prolonged milking interval (24 to 30h). Once daily milking impair tight junction function causing influx of SCC into milk, and neutrophils seem to continue to have an influx into milk even after twice daily milking has started again (Stelwagen and Lacy-Hulbert, 1996). According to Olde Riekering et al. (2007), if the time since last milking is less than 3 h, even healthy quarters can have a SCC close to 200,000 cells/ml. They found the geometric mean SCC increased until 7 h after milking. The explanation for this relied on a hypothesised high influx of cells shortly after milking, followed by dilution with large milk volume hours later. Irregular milking frequency (weekly coefficient of variation of MI > 22.7%) also decreased milk synthesis rate (Bach and Busto, 2005). By contrast, Weiss et al. (2002) found no differences in quarter SCC relative to udder filling when cows were milked at irregular intervals by AMS. It may be questioned if the irregularity affects only to the dynamics of SCC recruitment into milk or does it affect milk synthesis and udder physiology. However, it is likely that regular, but too short or too long MIs without any recovery would be even more detrimental for the udder.

Milking of a particular cow is permitted during AM according to different criteria. Permission for milking may be given, for example, on the grounds of the expected milk yield or time since last milking, or it may be counted based on the DIM and milk yield. However, the intended milking frequency may differ from that practised. On an experimental farm with AM, the average MI was 9.2 h, but 27% of the MIs were shorter than 6 h or longer than 12 h (Hogeveen et al., 2001). In a study of 8 commercial herds, approximately one third of MIs were shorter than 6 h or longer than 12 h (Gygax et al., 2007). Good management of AM includes assuring an adequate milking frequency for every cow according to its stage of lactation, instead of taking just the average milking frequency of the herd into account.

2.2.2 Fluency of the milking process

Kaihilahti et al. (2007) investigated 300 milking and cleaning processes on an AM farm and found that 5% of the milkings failed due to machine problems and 3% due to the cow. Only one third of all deviations were successfully compensated for by the robot. Other studies have shown consistent results with 8% and 4-5% of the milkings failing (Bach and Busto, 2005; Jago et al., 2006). Gygax et al. (2007) showed that attachment of the teat cup was successful in 94-98% of the milkings, depending on the AMS used, with some variation among the eight farms studied. An increased distance between the fore teats, especially in old cows, and a decreased distance between the hind teats in first parity cows, created most of the problems in the attachment of the milking cups (Miller et al., 1995). Incomplete emptying of the udder may lead to milk leakage (Stefanowskaja et al., 2001; Persson-Waller et al., 2003), discomfort of the cows (Stefanowskaja et al., 2001), impaired milk ejection in both affected and unaffected quarters (Bach and Busto, 2005),
and disturbed milking routine. Up to 9 milkings/day were recorded for cows with failed milkings (Klaas et al., 2008). Frequency of unsuccessful milkings increased from 5% to 30% during one week before clinical mastitis (Rasmussen et al., 2007), which could mean that clinical mastitis disturbed milking routine (or vice versa).

For milk ejection reflex to occur in time in AM, teat preparation is necessary, if milking starts immediately after attachment of the first teat cup (Dzidic et al., 2004a). Teat brushing decreased the dead milking time (time without detectable milk flow) for the three first attached quarters and the total cups-on time, compared with no teat brushing (Jago et al., 2006). Teat preparation of AMS has been shown to be sufficient for milk ejection (Bruckmaier et al., 2001; Hopster et al., 2002; Macuhova et al., 2003; Dzidik et al., 2004a), independent of the teat preparation method, i.e. brushing or cleaning with a cup with warm or cold water (Dzidic et al., 2004a; Dzidic et al., 2004b). With a low degree of udder filling after a short MI, or due to a low milk yield in late lactation, enough time for the teat preparation should be made possible by specific adjustments (e.g. requesting more than one brushing sequence) (Bruckmaier and Hilger, 2001; Dzidik et al., 2004a). Time from the first touch of the udder to the attachment of the last milking cup was considerably (3-4 times) longer for AM than for CM in an auto-tandem parlour (Gygax et al., 2007). On the other hand, protracted attachment in AM had no adverse effects on oxytocin release (Macuhova et al., 2004). Dzidic et al., (2004a), suggested that permission for milking could be based on the actual degree of udder filling (a proportion of milk yield compared to maximum storage capacity of the cow in month 2 of the respective lactation).

2.2.3 Teat condition

Quarter-based milking in AM reduces overmilking, compared with whole udder milking (Hogeveen et al., 2001), especially in the fore teats. Overmilking results in deteriorated teat condition (Hillerton et al., 2002). Some systems also provide a possibility for quarter pulsation, which might represent an opportunity to determine suitable settings for individual quarters, but to date no studies have assessed the effects of quarter pulsation on teat condition in AM. These kinds of properties of AM could reduce machine on-time for a quarter. Reduced machine on-time, especially at the end of milking when teats are getting empty, enhances maintenance of good teat condition (Rasmussen, 1993; Neijenhuis et al., 2000), which in turn could decrease incidence of clinical mastitis, (Neijenhuis et al., 2001b; Breen et al., 2009) because teat end callosity inhibits teat closure after milking and rough callosity ring may harbour mastitis bacteria (Neijenhuis et al., 2001b). On the other hand, with short MIs milk flow rate decreases and daily machine on-time becomes longer (Hogeveen et al., 2001). Milking 4 times/day negatively affected teat condition, because of short time left for teats to recover from previous milking (Ipema and Benders, 1992). As Neijenhuis (2001a) showed, milking can affect teats for much longer than usually believed; teat canal width was increased for up to 8 h after milking, and therefore MIs should not be too short in AM.

On 15 farms that changed from CM to AM, vascular disturbances of teats due to milking, teat skin condition score, and teat end callosity decreased after introduction of AM (Neijenhuis et al., 2004). The proportion of blue teats after milking increased however, but the reasons for this were not discussed. In studies comparing CM and AM, CM affected
teat thickness and length more than AM, but udder health remained good in both groups in a longitudinal study (Hamann and Schridde, 2005). The condition of the teat skin was worse in AM, but more vascular disturbances and teat end callosity was associated with CM (Berglund et al., 2002). Low prevalence of cows with high degree of teat end callosity was recorded on 8 AM farms (Klaas et al., 2008). Parity, season, variation in milk flow, failed milkings and milking frequency were associated with teat condition. Contradictory results were reported in Finland, where teat end callosity increased and teat skin condition deteriorated on 3 farms after the introduction of AM (Hovinen and Pyörälä, 2002). The negative effects decreased by increasing the switch-level of the AMS and changing the teat spray to promote a greater moisturising effect. A study comparing 40 cows that either changed from CM to AM or were always in CM, revealed no adverse effects for teat skin condition, but teat end condition was affected more in the heifers of AM group, and in the older cows in the CM group (de Vliegher et al., 2003). Differences in the results of the cited studies might be explained by the different AMS brands and study designs. In the study of Berglund et al. (2002), teats were prepared for milking both manually and automatically.

2.2.4 Bacterial ecology

Quarter-based milking in AM inhibits cross contamination of the teats with mastitis bacteria within a cow. However, transfer of bacteria between cows is possible through AMU. Using separate AMU for cows of different udder health status is possible only with a multi-robot system and several barn compartments. Traditional milking order, where infected cows are milked last, has been substituted in AM with rinsing the milking liners between cows. It is not known whether this short flushing is sufficient to stop the spread of bacteria in AM. In CM, back flushing of the liners was demonstrated to cut down the number of staphylococci by 98.5%, but it did not affect the incidence of clinical mastitis (Smith et al., 1985). In AM, back flushing with water removed 98.4% of \textit{Str. agalaktiae} from artificially contaminated liners, and none of the quarters of 46 cows milked with or without cluster flushing became infected (Schuiling, 2004). In contrast, after milking cows infected by \textit{S. aureus}, bacteria were detected from the liners of an AMS after a liner flush as often as in CM without liner flushing (Hovinen et al., unpublished). Currently, disinfecting liners between cows with heated steam has been introduced in AM. Regular changing of liners is as important in AM as in CM. This was demonstrated by de Koning et al. (2004), who showed that high BMSCC in AM was associated with using the same liners for too long, in that case twice as long as recommended.

Proportion of cow-days and individual cows leaking milk were higher in AM than in CM (Persson Waller et al., 2003). Milk leakage was recorded more frequently in primiparous cows (perhaps due to longer waiting times for the AMU), hind quarters, quarters with a high milk flow, cows lying down and soon after milking. Unsuccessful milking could also have resulted in some leaking of milk. Miltenburg et al. (2005) reported that milk leakage was recorded in 80% of the 600 Dutch farms included in a survey (farmer interview), which further emphasize the problem. Milk from infected quarters is a source of new infections for other cows (Elbers et al., 1998), but open teat canals also represent a route for potential IMI causing bacteria (Bramley et al., 1981; Hillerton, 1991), and milk leakage has previously been associated especially with \textit{E. coli} IMI (Schukken et al, 1991).
All AMS represent an opportunity for post-milking teat disinfection, but its reliability in covering the teats properly is debatable. Only half of the 42 cows studied had all their teat ends covered with teat spray from an AMU (Rasmussen and Hemling, 2002).

Distribution of bacteria isolated in IMIs in AM herds has not been exhaustively studied, and it is not known if it differs from that in CM herds. Some reports for individual AM farms exist. In Denmark, comparison among 18 farms before and after introduction of AM showed that prevalence of coagulase-negative staphylococci (CNS) increased (14% vs. 23%) in the older cows; in particular, incidence of subclinical IMIs caused by CNS was significantly higher during early and late lactation (Pedersen and Bennedsgaard, 2006). In the first parity cows the 2% increase in the prevalence of CNS was not statistically significant. Transfer of bacteria by the AMS teat-cleaning device was suspected to be one cause of the increased infections. Prevalence of S. aureus IMIs in the older cows was lower after introduction of AM, but the difference was not statistically significant. In three Finnish AM farms (Hovinen and Kasanen, 2004), CNS caused most of the mastitis cases (SCC > 200 000 cells/ml), but environmental bacteria (Str. uberis, Str. dysgalagiae, Klebsiella pneumonia, E. coli and Enterococcus sp.) caused three times more mastitis than what was observed in a Finnish survey (Pitkälä et al., 2004), although more clinical mastitis and loose housing must have been in the material of AM herds. In three German AM farms, environmental streptococci and CNS were the main causes of subclinical IMI (Petermann et al., 2002). In a pasture-based system Str. uberis and CNS were most commonly isolated in both AM and CM (Lopez-Benavides et al., 2006).

In an AM herd where the prevalence of S. aureus IMI at calving was 3% of the cows, infection spread to 67% of the cows during the one year study period (Zecconi et al., 2004). In a herd of about 100 cows, 15 developed coliform mastitis, most of which was caused by Klebsiella spp. (Tuiskunen et al., 2006). Bacteria were isolated from several sites of the AMU and from stalls where an infected cow had been lying. A similar case occurred in Sweden, where Klebsiella bacteria were transferred to the cows, possibly by contaminated teat cleaning brushes of the AMU (Persson Waller and Unnerstad, 2004). Wendt and Pallas (2004) studied two AMS herds and suggested that sub-optimal cleaning and disinfection of the AMUs (brushes, liners and the multipurpose arm), as well as insufficient post-milking disinfection of the teats, resulted in high mastitis prevalence in the herds. These findings highlight the importance of proper AMU management because it can be responsible for the spread of infections among cows.

2.3 Teat cleaning in automatic milking

Good milking hygiene is essential to maintain milk quality. Thorough teat cleaning before milking reduces the number of bacteria on the teat skin and consequently in the milk (Pankey, 1989). Milking hygiene also has an impact on udder health of the cows because pathogens can enter the teat canal during milking if conditions are suboptimal, especially during overmilking (Thiel et al., 1969; Rasmussen et al., 1994). Evidence of an association between teat or udder contamination with manure or the number of mastitis bacteria on teat ends and mastitis was reported (Zarkower and Scheuchenzuber, 1977; Bramley et al., 1981; Pankey, 1989). Cleaning of the udder and teats using water without
drying was associated with high cow SCC (Köster et al., 2006). Klebsiella spp. were found from teat skin more often after teat cleaning of cows with dirty udders, and the proportions of different subspecies found on the teat skin were similar to those found in milk from quarters with Klebsiella mastitis (Munoz et al., 2008). Experimental IMI caused by Str. uberis was reduced by teat preparation compared with no teat preparation (Galton et al., 1988). Bartlett et al. (1992) demonstrated an association between the prevalence of Str. agalactiae IMIs and teat and udder hygiene. Poor udder and leg hygiene was associated with a high cow SCC (Schreiner and Ruegg, 2003; Reneau et al., 2005), clinical mastitis incidence (Breen et al., 2009) and incidence of mastitis caused by environmental and contagious bacteria (Schreiner and Ruegg, 2003). The authors suggested that the reason for more IMI caused by contagious bacteria was that teat dipping was not efficient for dirty udders (Bartlett et al., 1992; Schreiner and Ruegg, 2003). According to European Union legislation, milking must be carried out hygienically and before milking teats, udders and the adjacent areas must be clean (Regulation (EC) No 853/2004).

AMU cleans the teats with automatic devices. No method is available to distinguish between dirty and clean teats before cleaning, nor for monitoring the effectiveness of the cleaning (Mottram, 1997). However, such methods have been investigated (Bull et al., 1995, optical inspection system; Mottram and Persaud, 2000, olfactory system; Ordolff, 2005, image processing system). Not all of the current AMS have sensors to detect whether a teat is in the cleaning device during cleaning or if the teat is actually cleaned. The effective operation of the AMS is crucial because in AM the result of teat cleaning no longer depends on the careful attention of the milker.

Limited research on the effectiveness of teat cleaning (ETC) and the technical success of teat cleaning (TSTC) in AM is available. Eight percent of the teat cleanings per cow failed due to machine problems and 4% because of cow related problems, including kick-offs (Kaihilahti et al., 2007). Jago et al. (2006) observed 130 teat cleaning periods in AM and found that only 67% of the cleanings were technically successful, i.e. all 4 teats were brushed. In another study, approximately 10% to 20% of the teat cleanings per cow failed technically (Hvaale et al., 2002). Some studies have been published on the ETC of AM under experimental (Schuiling, 1992; Melin et al., 2002; Knappstein et al., 2004) or field conditions (Ten Hag and Leslie, 2002; Knappstein et al., 2004; Tangorra et al., 2004; Bade et al., 2008). Most agree that teat cleaning in AM is not as effective as in CM.

2.4 Mastitis detection in automatic milking

2.4.1 Detection of subclinical mastitis

Subclinical mastitis is inflammation of the mammary gland, almost always caused by an infecting microorganism, which is not visible and requires a diagnostic test for it to be detected (IDF, 1999). Milk SCC is the most used diagnostic test for subclinical mastitis (IDF, 1999). Cow milk SCC of > 200 000 cells/ml indicates mastitis (IDF, 1997; Hillerton 1999; Schukken et al., 2003). Recently it has been suggested that SCC is always below
100 000 cells/ml in a healthy quarter (Hamann et al., 2002). Mastitis should be detected in a reliable and timely fashion (Pyörälä, 2003) otherwise subclinical mastitis can develop into a clinical disease (Hallén Sandgren et al., 2008). Cows with undetected mastitis also represent an infection risk for other cows. Composite milk SCC of > 200 000-300 000 cells/ml should be detected to initiate preventive or therapeutic measures (Hamann and Zecconi, 1998). It is not known if subclinical mastitis represents a welfare problem for the cow, causing pain or discomfort, but the mechanical threshold to pain related to mastitis was reported to be lowered also in cases of mild clinical mastitis, without local or systemic signs (Kemp et al., 2008).

In AM different types of on-line device have been installed for detecting subclinical mastitis. Most of them are based on measuring characteristics of milk continuously and repeatedly from the milk line. This enables the use of different mathematical methods to process the data: inter-quarter comparison of the values, calculating running averages of the parameters (Maatje et al., 1992), analysing patterns of milk variables during milking, picking up maximum/minimum values during milking etc. (Norberg et al., 2004). On the other hand, some characteristics of AMS may complicate the use of the data: measurement errors or missing data, unequal numbers of observations within milking between cows, and the dependency on subsequent measurements within and between milkings (Nielen et al., 1995). In addition, using multivariate methods with external information about the cow combined as intelligent knowledge-based methods and decision-making systems have been suggested (Hogeveen et al., 1991, Asseldonk et al., 1998; de Mol et al., 2001; Steeneveld et al., 2008).

### 2.4.2 Detection of clinical mastitis

Clinical mastitis is intramammary inflammation (mostly caused by infection), characterised by visible abnormalities in the milk, in the udder, or in both, and can be classified as mild, moderate or severe (IDF, 1999). In severe mastitis the general condition of the cow is affected. All cases of clinical mastitis should preferably be detected before clinical signs become severe, in order to be able to start the therapeutic measures early enough to counter the infection (Milner et al., 1997). Early detection of clinical mastitis in AM depends on the order of events; for example, *E. coli* mastitis could proceed too quickly to allow treatment decisions to be made before development of clinical signs in twice daily milking (Nielen et al., 1992). In studies with experimentally induced *Str. uberis* and *S. aureus* (Milner et al., 1997), and *Str. uberis* (Hillerton and Walton 1991; Lake et al., 1992), clots in milk were detected one day after the increase in electrical conductivity (EC). On the other hand, to be useful, an alert should be given at the first milking when clinical mastitis is present or during a very limited time before (Kamphuis et al., 2008). Milk from cows with clinical mastitis can contain high numbers of bacteria, and may transmit the infection to other cows through stalls or via milking equipment. Severe clinical mastitis can also result in a blind quarter. Clinical mastitis is a painful condition and the cow should be treated as fast as possible to support the elimination of bacteria from the udder quarter, to control pain and inflammation, and to prevent further damage to the udder tissue.
EU legislation (Regulation (EC) No 853/2004) states that milk has to come from animals without signs of clinical mastitis. Consequently, AMS should be able to separate abnormal milk automatically. To avoid separating normal milk, the evaluation method should be highly specific for healthy quarters, with high positive predictive value (PPV), and highly sensitive to mastitic quarters. In the ISO-standard for automatic milking installations (ISO/DIS 20966, 2007), methods for automatic detection of abnormal milk should have sensitivity (SE) > 70% and specificity (SP) of > 99%. In general, SE and SP of the evaluation methods for automatic separation of milk have been claimed to be too low (Rasmussen, 2004). In tests performed according to the guidelines of the ISO-standard, SEs for automatic separation of milk have ranged from 28% to 45% for abnormal milk (>2 mm visible clots in milk with CMT score > 3) and 100% for milk with blood (>2% of blood in milk), depending on the AMS model (Rasmussen, 2006). Detection methods used in AM could also be used for optimising the BMSCC, as was experimentally done by Graupner et al. (1992), who tested different EC thresholds on separation of quarter milk and recorded the highest possible BMSCC with the lowest possible amount of milk separated. In AM, milk from the affected quarter is not separated alone as in CM, but if technically possible, it could be preferable for economic reasons (Forsbäck et al., 2009).

2.4.3 Methods for mastitis detection in automatic milking

2.4.3.1 Electrical conductivity

EC is the most common indicator used to detect subclinical and clinical mastitis in the current models of AMS. On-line method for measuring EC was developed in the eighties (Lake, 1987; Rossing et al., 1989). The measurement is based on the increase in Na\(^+\) and Cl\(^-\) in the mastitic milk, due to the inflammation of the udder (Hamann and Zecconi, 1998). During inflammation, permeability of the capillaries increases, allowing Na\(^+\) and Cl\(^-\) to pass from blood and extracellular fluid to milk through leaky tight junctions between the epithelial cells. Cells can be damaged as the disease progresses, causing destruction of the ion-pumping system (Kitchen et al., 1980). Na\(^+\) and Cl\(^-\) diffuse into the milk and K\(^+\) into the extracellular fluid to maintain the osmotic pressure of the milk (Kitchen, 1981). In clinical mastitis, the increase in milk SCC has been shown to last longer than the increase in EC, suggesting that the ions may be able to pass through the tight junctions into milk only when the rate of SCC diapedesis is high (Bruckmaier et al., 2004).

According to several studies, milk EC measurement is inadequate to detect subclinical mastitis (Nielen et al., 1992; Hamann and Zecconi, 1998; Biggadike et al., 2002; Nordberg et al., 2004\(^a\), Bruckmaier et al., 2004) (Table 2). EC of milk shows only a slight change at the levels of SCC from 200 000 to 300 000 cells/ml (Hamann and Zecconi, 1998). Nielen et al. (1993) showed that the correlation between EC and lnSCC was not satisfactory, although both were related to depression of milk yield. However, the state of udder cell destruction may be better described by EC than by SCC, at least in the cows without clinical mastitis (Nielen et al., 1993). In a meta-analysis of 17 studies, SE of milk EC was its highest when positive bacteriology was used as the gold standard for subclinical
mastitis (Nielen et al., 1992), but controversially, the definition of healthy quarters as being those without infection was found inadequate in Sloth et al. (2003), and number of false positive (FP) and false negative alerts was lower when SCC instead of IMI was used as a gold standard (Lansbergen et al., 1994). Correlation coefficients between EC and milk SCC have been reported to be from 0.52 (foremilk) to 0.58 (strip milk) (Bansal et al., 2005) and from 0.37 to 0.47 (median of 16 studies) (Nielen et al., 1992).

As for subclinical mastitis, milk EC measurement has been concluded to be inadequate also for the detection of clinical mastitis (Hamann and Zecconi, 1998) and abnormal milk in general (Rasmussen, 2004) (Table 2). However, EC can be useful in providing an early alert before clinical signs of mastitis appear (Lake et al., 1992; Maatje et al., 1992; Milner et al., 1997). For example, in Maatje et al. (1992), nearly 65% of cows with naturally occurring clinical mastitis had an alert before clinical signs appeared. Milner et al. (1997) were able to prevent development of clinical signs of experimentally induced Str. uberis and S. aureus mastitis in 3 out of the 8 cows in both groups by administering antibiotic treatment as soon as the milk EC increased.

EC of milk is dependent on many cow and herd related factors (Hamann and Zecconi, 1998). The size of the fat globules and the structure of casein affect EC (Mabrook, 2003). Electrolytes reside in the liquid phase of the milk and fat globules increase the distance the ions have to travel and reduce the volume of the conducting medium (Prentice, 1962). Milk composition is further influenced by feeding and breed (Hamann and Zecconi, 1998). Lactation stage affects EC through hormonal status, blood circulation, intramammary pressure and integrity of the tight junctions. For example, oestrus affects the Na\(^{+}\)-pump through oestrogen (Hamann and Zecconi, 1998). Norberg (2004\(^{b}\)) found that EC of the milk was highest post partum, then decreased until 50 DIM and rose during the end of the lactation. Temperature of milk from healthy glands has been shown to affect milk EC by 0.12 mS/cm/1°C milk and that from glands with clinical mastitis has twice the effect (Pachauri 2000). Electrode quality and design influence milk EC (Lake et al., 1992) because dirt, milkstone, fat and protein can cover the electrodes. Clots in milk interfere with electrodes, and milk flow stoppages cause variation in EC pattern during milking between normal and abnormal milk (Norberg et al., 2004\(^{a}\)).

EC of the milk has been shown to be highest in milk obtained before the first touch of the udder, i.e. before the milk ejection reflex in the cisternal milk, and decreasing in the alveolar milk fraction (Woolford et al., 1998; Bruckmaier et al., 2004; Bansal et al., 2005). Bruckmaier et al. (2004), by repeatedly collecting milk samples from manually stimulated teats, showed that foremilk is cisternal for only 40 seconds after the first touch of the udder. After that cisternal and alveolar milk mix. Cisternal milk is of the best diagnostic value in the detection of mastitis (Woolford et al., 1998; Bruckmaier et al., 2004). The theory behind this finding is not clear; Woolford et al. (1998) suggested that IMI in the cisternal region might cause local leakage of ions, or leakage of milk with a high EC from infected regions of the alveolar region between milkings. Fat content of the milk is lowest in cisternal milk (Weiss et al., 2002) and increases towards the end of the milking (Woolford et al., 1998; Bansal et al., 2005), causing EC to decrease towards the end of the milking (Hamann and Gyodi, 1999; Bansal et al., 2005). On the other hand, fat-corrected EC increases towards the end of the milking, but does not reach levels as high as in the cisternal milk (Woolford, 1998; Barth and Worstorff, 2000), although controversial results
exist (Fernando et al., 1981). EC of the strip milk was shown to be affected by udder health status and bacterial species responsible for the IMI in the quarter (Hillerton and Walton, 1991; Woolford et al., 1998; Bansal et al., 2005) but conclusions from the studies are not consistent.

In AM, EC is measured after the beginning of milk ejection, when the first millilitres of milk are discarded, and measuring continues throughout the milking. Consequently, milk from quarters with subclinical mastitis is given too low an average value of EC per milking. Hamann and Gyodi (1999) suggested that at least a difference corresponding to > 200 000 cells/ml from the normal level is needed to detect mastitis if EC measurement after milk ejection is used. Bansal et al. (2005) therefore suggested measuring EC from the strip milk in the on-line systems. Nordberg et al. (2004a) however showed that quarters with clinical and even subclinical mastitis can have totally divergent EC patterns during the milking. Woolford et al. (1998) suggested analysing EC from different milk fractions, which could also provide information on the infective agent.

EC has been shown to be lowest after a MI of 9h to 12h (Fernando and Spahr, 1983; Hamann and Gyodi, 1999; Barth and Worstorff, 2000). An increase in EC after a MI of 14h (Hamann and Gyodi, 1999) or 15h (Fernando and Spahr, 1983) was suspected to be due to a lower fat content and a higher chloride concentration in the milk, compared with milk after shorter MIs (Hamann and Gyodi, 1999), or a higher intramammary pressure, which may increase permeability of the secretory epithelium (Fernando and Spahr, 1983). In the future MI should be taken into account in applying EC to mastitis detection in AM (Barth and Worstorff, 2000).

Table 2. Performance of electrical conductivity in detecting mastitis in conventional (CM) and automatic (AM) milking. Data compiled from different sources.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Material</th>
<th>Definition of mastitis</th>
<th>SE¹ %</th>
<th>SP² %</th>
<th>PPV³ %</th>
<th>NPV⁴ %</th>
<th>Special remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subclinical mastitis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maatje et al., 1992</td>
<td>68 quarters</td>
<td>SCC &gt; 500 000</td>
<td>52-53</td>
<td></td>
<td></td>
<td></td>
<td>CM, on-line</td>
</tr>
<tr>
<td>Nielen et al., 1992</td>
<td>17 studies SCC, IMI or both</td>
<td>median 66 (6-100)</td>
<td>median 94 (0-100)</td>
<td></td>
<td></td>
<td></td>
<td>CM, meta-analysis</td>
</tr>
<tr>
<td>Biggadike et al., 2002</td>
<td>31 cows SCC &gt; 200 000 or 400 000</td>
<td>40-54</td>
<td>85-92</td>
<td>33-55</td>
<td>87-93</td>
<td></td>
<td>AM</td>
</tr>
<tr>
<td>Norberg et al., 2004</td>
<td>322 cows IMI</td>
<td>3-16</td>
<td>92-98</td>
<td></td>
<td></td>
<td></td>
<td>CM, on-line</td>
</tr>
</tbody>
</table>
Clinical mastitis (Table 2 continued)

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Description</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Error rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maatje et al., 1992</td>
<td>25 cases</td>
<td>clinical signs</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norberg et al., 2004</td>
<td>322 cows, 275 cases</td>
<td>treatment, clinical signs</td>
<td>16-46</td>
<td>92-98</td>
<td></td>
</tr>
<tr>
<td>Hamann and Zeconni, 1998</td>
<td>6 papers, 17 datasets</td>
<td>clinical signs/SCC &gt;500 000 + IMI/SCC &gt; 1 mill.</td>
<td>68</td>
<td>82</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment/ cow SCC &gt; 100 000, treatment/ cow SCC &gt; 400 000</td>
<td>84-88, 85-86</td>
<td>67-73, 75-82</td>
<td>56-60, 83-87</td>
</tr>
<tr>
<td>Cavero et al., 2007</td>
<td>160 cows, 1 year</td>
<td>treatment/ cow SCC &gt; 100 000, treatment/ cow SCC &gt; 400 000</td>
<td>84-88, 85-86</td>
<td>67-73, 75-82</td>
<td>56-60, 83-87</td>
</tr>
</tbody>
</table>

1 SE sensitivity
2 SP specificity
3 PPV positive predictive value
4 NPV negative predictive value

2.4.3.2 Milk colour

Milk colour changes during clinical mastitis and physical damage of the udder as blood constituents leaks from the vessels. The definition of subclinical mastitis describes milk without visual changes (IDF, 1999). However, Wiedemann and Wendl (2003) reported good results in detecting subclinical mastitis with a spectrophotometric method, which accounted for the influence of milk fat on the colour of the milk. In contrast, Ordolff (2002) reported that based on the colour of foremilk, detection of subclinical mastitis was not satisfactory.

A sensor measuring reflected light intensity can be used to measure milk colour and detect abnormal milk (Ouweltjes and Hogeveen, 2001; Espada and Vijverberg, 2002) and blood in the milk (Ouweltjes and Hogeveen, 2001). This colour sensor analysed a continuous flow of milk in AM and detected blood in the milk at concentrations as low as 0.1%. The conventional visual method using a black strip cup, detected only minimum of 2.0% of blood in the milk (Rasmussen and Bjerring, 2005). Green and blue colours were the best indicators for abnormal milk and clinical mastitis, although they did not correlate well with the appearance of the milk (Kamphuis et al., 2008b). Whyte et al. (2004) reported a high SE and SP for detecting blood using an optical in-line sensor.
2.4.3.3 SCC

Milk SCC is the most used indirect indicator of subclinical mastitis (Schalm, 1971; IDF, 1999, Hamann, 2002). According to results from a number of studies (Harmon, 1994, Pyörälä, 2003), the effect of lactation stage, parity and season have a minor influence on milk SCC of healthy quarters, compared with infection status of the quarter and MI. In AM, on-line SCC measurement is a new method for the detection of subclinical mastitis. Two systems for on-line SCC analysis of milk are currently available:

1) A direct method: dyeing the nuclei of somatic cells and counting them automatically from a photo. Milk samples are taken from the cow composite milk.

2) An indirect method: hydrolysing the DNA of the nuclei of somatic cells and measuring the viscosity of the compound. The system gives estimates of SCC similar to a CMT test. The milk sample is taken from the first fraction of the milk after milk ejection has started. This method operates at quarter level.

There are few publications currently available on the efficacy of these methods to detect mastitis in AM. Mollenhorst et al. (2008) compared indirect on-line quarter milk SCC data with quarter milk SCC determined in a laboratory, and with visual detection of abnormal milk. Correlation between on-line SCC and laboratory SCC was 0.51. SE and SP for detecting visually abnormal foremilk with on-line or laboratory SCC, at a threshold of 500 000 cells/ml, were nearly similar (SE 55% and 50%, SP 94% and 92%), corresponding well to the results of Rasmussen et al. (2005), using CMT score 4 to 5 as an indicator of abnormal milk (with SE 58%, SP 88%). Differences between laboratory and on-line SCC measurements could be due to different fractions of the milk being used: milk during the first 30 seconds of milking (on-line SCC) compared with quarter composite sample (laboratory SCC). Decrease in SCC from cisternal to alveolar milk fractions was 80% for milk from a quarter with a high SCC (Sarikaya and Bruckmaier, 2006). However, usually and in the study of Mollenhorst et al. (2008) when foremilk samples are taken after milk ejection, they should be quite representative of the quarter composite milk, at least with SCC 50 000-300 000 cells/ml (Wellnitz et al., 2009).

Overall correlations on cow composite milk SCC between SCC analysed in a laboratory or with the indirect on-line SCC method of AMS (method described by Whyte et al., 2004) were 0.76 (Kamphuis et al., 2008) and 0.71 (Leslie et al., 2007), and for quarters with SCC > 200 000 cells/ml, 0.82 (Kamphuis et al., 2008). Respective numbers for quarter EC and cow SCC analysed in a laboratory were 0.48 and 0.33 (Kamphuis et al., 2008). Of quarters having > 200 000 cells/ml analysed in a laboratory, only 77% also had an SCC of > 200 000 cells/ml analysed with an on-line SCC detector. Mathematical models of mastitis detection in AM using either EC or on-line SCC measurement had as high an SE for detecting 20 quarters with clinical mastitis as a model which combined the two parameters (Kamphuis et al., 2008). However, an on-line SCC model gave fewer FP alerts than an EC model, and combining the models decreased the number of FP alerts even more. Results of Poskiparta (2009), who interviewed 27 farmers who used on-line SCC detection in AM, claimed that the SCC results correlated well with the test day SCCs analysed in the laboratory. Farmers also trusted the results of on-line SCC measurement more than the results of the EC measurement. Of course this is a subjective perception of the farmers and cannot be used as scientific evidence. Moreover, only 30% of the farmers responded to the interview.
2.4.3.4 Milk yield

Acute clinical mastitis results in a sudden drop in milk production (Lake et al., 1992). Changes in milk production and flow rate can be of value in identifying mastitic quarters (Maatje et al., 1992; Kohler and Kaufmann, 2002). Milk yield decreased already one week before the treatment of more than 900 cases of clinical mastitis, and started to increase within 3 weeks post-treatment (Rasmussen et al., 2007). Alerts are usually created in AM on the basis of declining milk yield, but they are not necessarily integrated with the methods for mastitis detection.

2.4.3.5 Infra-red thermography

Infra-red thermography (IRT) is a non-invasive method for measuring radiated heat emitted by the skin that reflects subcutaneous circulation and metabolism (Jones and Plassmann, 2002). Radiated heat emitted by the udder during clinical mastitis can be detected with IRT. IRT is not related to milking or milk itself, which provides a possibility for detecting mastitis during the dry period and before the first calving.

Berry et al. (2003) developed a predictive model for the temperature of the udder surface based on consecutive measurements of healthy cows and ambient temperature. They concluded that IRT showed promise for early detection of mastitis. IRT was not suitable for detection of subclinical mastitis (Barth, 2000). Preliminary studies on the detection of clinical mastitis using IRT have shown promising results (Hurnik et al., 1984; Scott et al., 2000; Kemp et al., 2008; Metzner et al., 2008). A thermal camera installed in an AMS milking or feeding parlour could generate alerts already before milking a cow with clinical mastitis.

2.4.3.6 Other methods

Milk temperature reflects body temperature (Lira et al., 1975; Maatje and Rossing, 1976) and has been measured in some AMS models. Air leakage into the milking cup, time from the start of milking, ambient temperature (Lira et al., 1975) and milk flow rate (Maatje and Rossing, 1976) may affect milk temperature measured from the clawpiece. Milk temperature has, however, been included in detection models for mastitis (de Mol et al., 1997, de Mol et al., 1999), with a conclusion that it had additional value in the model (de Mol and Ouweltjes, 2001).

New sensor technologies such as measuring volatile components (Eriksson et al., 2005; Hettinga et al., 2008) and potentiometric values of milk have been studied (Mottram et al., 2007). A newly invented biosensor system that analyses lactose and EC has been claimed to have a SE of over 90% in identifying quarters with ≥ 100 000 cells/ml, irrespective of the type of mastitis (Culina et al., 2006). Measurement of milk hydrogen peroxide degradation by catalase (associated with mastitis bacteria), has been also been suggested for detection of mastitis (Leth et al., 2004).
In the future, measurement of particles in the milk may be used (ISO/DIS 20966, 2007). Hillerton and Walton, (1991) detected clots with an on-line clot detector from quarters with experimental *Str. uberis* mastitis one day before visual detection of clinical mastitis. An optical device detecting flakes with a diameter ≥ 0.1 mm and classifying the objects found is under development (Maassen-Franke et al., 2004).

Milk N-acetyl-β-D-glucosaminidase (NAGase) activity measurement has been proposed as a method for detecting clinical mastitis in AM (Mottram et al., 2000). Milk NAGase is a lysosomal enzyme of epithelial cells and white blood cells of the udder that is released in milk during mastitis (Kitchen, 1978). On-line sampling of milk and analysis of L-lactate dehydrogenase incorporated into a decision support model with EC and other relevant data are currently being used commercially to calculate the risk of clinical mastitis and a relative degree of chronic mastitis. The SE for detecting clinical mastitis using this method was 82% and the SP 99% (Chagunda et al., 2006). Indicators of acute phase reaction like serum amyloid A and haptoglobin in milk have also been studied as potential indicators of mastitis, but some variation in their concentrations were recorded during subclinical mastitis (Grönlund et al., 2005). A significant relationship was established between quarter and cow SCC and haptoglobin and serum amyloid A of clinically healthy cows (Åkersted et al., 2007). A near-infrared spectroscopic on-line system for determining different milk constituents including SCC has recently been investigated with promising results for subclinical mastitis (Kawasaki et al., 2008).

### 2.4.3.7 Multivariate methods

In addition to the primary detection methods for mastitis, multivariate methods, combining e.g. milk yield, milking frequency and cow activity data, together with measurements from milk, have been suggested as being useful (Hogeveen et al., 1991; Asseldonk et al., 1998). Using multivariate methods or fuzzy logic in interpreting data would improve chances for separating healthy quarters from quarters with clinical (de Mol and Ouweltjes, 2001; de Mol and Woldt, 2001) or subclinical mastitis (Nielen et al., 1995; Sloth et al., 2003). Nielen et al. (1992) suggested a multivariate approach for mastitis detection, possibly a sequential model based on a meta-analysis of studies on EC. A few years later, Nielen et al. (1995) studied different models for interpreting results from on-line EC measurement combined with milk temperature and yield data, with mean SE 63-80% and SP 78-100% for two milkings before clinical signs of mastitis. They further improved the method using a neural network technique, which detects patterns irrespective of data distribution, missing data or abnormal values, and can distinguish between healthy quarters and those with clinical mastitis (Nielen et al., 1995).

Multivariate time-series models using EC, milk temperature and milk yield with a Kalman filter (a method to estimate the state of a system on-line) have been developed for subclinical (de Mol et al., 1997; de Mol et al., 1999) and clinical mastitis detection (de Mol et al., 1997; de Mol et al., 1999, de Mol et al., 2001) in CM. A multivariate model using EC and milk yield was also created for AM (de Mol and Ouweltjes, 2001). The model became accustomed to a certain level of variables (de Mol et al., 1999), which could be both an advantage and a disadvantage in practice. With a fuzzy logic model for classifying alerts of clinical mastitis as true or false based on EC, and additional
information about the cows (de Mol and Woldt, 2001), the number of FP alerts, which was too high in earlier models for practical conditions, could be reduced. In field tests, a model for detecting illnesses (using cow activity instead of EC) had higher SE for detecting clinical mastitis than an actual mastitis detection model (de Mol et al., 2001). The SE and SP reached 80% and 98.6% (at a confidence level of 95%). Information about milk temperature, lactation curve, number of visits to the AMU, concentrate leftovers and previous incidents of mastitis should be included in future models (de Mol and Ouweltjes, 2001).

Cavero et al. (2006) developed a multivariate model to detect mastitis in AM that incorporated EC, milk yield, milk flow rate and MI. The model used a fuzzy logic support method to aid decision-making that classified results as indicating mastitis, different degrees of likelihood for mastitis, or no mastitis. The results suggested that more informative parameters would be necessary to improve the model. A method using neural networks to imitate brain function with learning abilities was later tested. It used variables including EC, DIM, milk production rate and milk flow rate, but the results were inferior in comparison with the earlier ones (Cavero et al., 2008). Using a combination of EC and spectral reflectance measurement in milk, Wiedemann (2004) reported a SE of 73% for detecting quarters with SCC > 500 000 cells/ml and 85% for detecting of quarters with > 500 000 cells/ml and an IMI.

Variables describing the shape or variability of EC, milk production rate and milk colour for detecting quarters with abnormal milk or clinical mastitis were tested in AM (Kamphuis et al., 2008b). Absolute EC values were important, even though more sophisticated methods use deviation from expected values. Variability and shape of measurement patterns were as important as variables describing the mean or maximum. Variables did not correlate well with milk appearance. The authors suggested different approaches for the detection of abnormal milk and clinical mastitis, but this can be disputed because abnormal milk is a result of clinical mastitis (IDF, 1999). Models should comprise combinations of different variables, different types describing the pattern of a variable, comparison with previous measurements, comparison between quarters, and use of different time-frames (Kamphuis et al., 2008b). Subsequently, with promising results, they also used EC, milk production, dead milking time and milk flow in AM with a decision-tree induction that could handle noisy, imbalanced and incomplete data (Kamphuis et al., 2008a). They also combined on-line SCC and EC measurement in a fuzzy-logic model and were able to decrease the false alert rate and increase the success rate of clinical mastitis detection 2 to 3-fold compared with EC or SCC measurement alone (Kamphuis et al., 2008a). Hassan et al. (2009) reported good results in classifying quarters into non-infected, infected with minor bacteria or infected with major bacteria, by analysing quarter SCC and EC among others and taking advantage of a neural network technique in CM. Steeneveld et al. (2009), classified mastitis bacteria as gram- or gram+ using cow data that could be made available in AMS. These highly advanced methods should be developed into commercial solutions in AM in the near future.
3 AIMS

1. To study the effect of change in barn type and milking system on udder health of cows transferred from tie stalls or free stalls with CM to free stalls with either CM or AM. To establish whether the reasons for the recorded higher BMSCC in the AM herds, compared with the CM herds in Finland, is due to inferior udder health in AM.

2. To evaluate the technical success of teat cleaning and the effectiveness of teat cleaning in AM under field conditions.

3. To examine management factors in the dairy herd to improve teat cleaning in AM.

4. To study the performance of methods of AM for detection of clinical and subclinical mastitis and the reliability of alerts under field conditions.

5. To improve the performance of EC in AM with different criteria for EC alerts in existing systems.

6. To test a non-invasive IRT method for detection of acute clinical mastitis.

Figure 1. Aspects of automatic milking studied in this thesis.
4 MATERIAL AND METHODS

4.1 Farms and study animals (I-IV)

A total of 182 Finnish dairy farms that changed their cattle housing or milking system or both were included in study I. Of 88 AM herds 29 were housed in tie stall barns and 59 in free stall barns with CM before the change. Of 94 CM herds, all were housed in tie stalls with CM before the change into free stall barn. Mean (median) herd size for the AM group was 38 (36) cows before and 45 (42) cows after the change (cows in milk). Respective figures for the CM group were 24 (22) and 34 (32). Mean (median) lactation number for the AM group was 2.1 (2.0) before and 2.0 (2.0) after the change, and for the CM group 2.3 (2.0) and 2.2 (2.0), respectively. The predominant breeds were Finnish Ayrshire (71%) and Holstein Friesian (29%).

In studies II and III, 9 (study II) and 8 (study III) dairy herds that had been milked automatically for a minimum of 6 months and had only 1 automatic milking stall were included. Herds were divided into Group A (5 and 4 herds in studies II and III respectively) and group B (4 herds) based on the brand of the AMS used on the farm. Group A consisted of 161 cows and 616 teats and Group B of 184 cows and 716 teats in study II, and 112 cows and 430 teats in Group A and 171 cows and 655 teats in Group B in study III. In both studies Group A cows were older ($P < 0.001$), had a higher milking frequency ($P < 0.05$) and were later in lactation (only in study III) ($P < 0.001$).

Study IV was an experimental study. Five Finnish Ayrshire cows and one Holstein-Friesian cow were experimented on; 5 cows were in their first lactation and one cow in her second lactation. Cows were housed in a tie-stall barn of 68 milking cows in stalls bedded with wood shavings. They had free access to good quality silage and water, and were fed with concentrate 6 times daily according to their state of lactation. The cows were milked twice daily with a pipeline milking machine (DeLaval Harmony®, DeLaval International AB, Tumba, Sweden) at 05.30 and 17.30. Milk yield of the cows was 26.7 kg/d (standard deviation 6.9 kg). They were in late lactation with an average of 190 DIM (standard deviation ±34, minimum 155, maximum 249 DIM). Cows were clinically healthy, with all experimental quarters free from bacterial growth and SCC < 100 000 cells/ml.

4.2 Udder health data (I)

The Agricultural Data Processing Centre Ltd. provided milk record data from 1999 to 2006. The data included herd size per year, parity, breed, calving dates, test day data (date, milk yield and cow SCC) and records of mastitis treatments. Data comprised 140 327 cow-test milkings, 70 453 composite SCC samplings (at least every other month from one milking on the test day) and a total of 2 989 mastitis treatments.
4.3 Clinical examination of cows and classification of severity of mastitis

In study III all cows and quarters were milked once in the presence of the investigators during autumn 2003. Foremilk was separated after teat cleaning by the AMS for Group A and manually for Group B. Before milking, milk was collected for SCC determination and assessment of appearance. The udder was palpated and a sample for bacterial analysis was taken after milking.

Farmers kept records of cases of clinical mastitis for 6 months after the visit. In addition, the farmers were advised to examine all cows that had an alert over a 15-day to one month follow-up period. Examinations included CMT scoring, visual inspection of the milk and udder palpation.

Clinical examination of the cows in study IV included recording the rectal temperature ($T_R$), and evaluation of the systemic signs of the cow, local signs of the udder and milk appearance.

4.3.1 Classification of subclinical mastitis (III)

SCC limits of 200 000, 400 000 and 1 000 000 cells/ml milk represented mild, medium and severe case of subclinical mastitis. The limit of 200 000 instead of 100 000 cells/ml was selected because it is considered to be an operational threshold of practical value under field conditions (Schukken et al., 2003), minimising diagnostic errors.

4.3.2 Classification of clinical mastitis (III and IV)

Quarters with abnormalities in the milk in study III were divided in 3 categories:
1) clinical mastitis: changes in the appearance of the milk and CMT $\geq 3$
2) bloody milk: milk with red colour
3) colostrum: yellow milk, DIM $\leq 4$ days, CMT $\geq 3$

Clinical signs of the cow were assigned to 3 categories in study IV (modified from the system of IDF, 1999):
1) normal: attentive cow with good appetite (systemic status); udder not swollen (local status); white and homogeneous milk (milk status)
2) mild to moderate: depressed cow with decreased appetite (systemic status); udder slightly swollen (local status); slightly discoloured milk or milk containing small flakes (milk status)
3) severe: apathetic cow with anorexia (systemic status); painful and very swollen udder (local status); strongly discoloured, watery milk or milk containing large clots (milk status)
4.4 Teat cleaning devices (II)

Group A used a separate cleaning cup that cleaned the teats with warm water, variable air pressure and vacuum, and dried the teats with warm air. The length of the cleaning process was normally 12 s/teat for each cow. Teats were located by lasers and a camera before cleaning. Group B used wet rotating brushes to clean the teats from apex to base and back. After every cleaning between cows the brushes were sprayed with warm water and disinfectant. The teats were located based on udder coordinates defined at earlier milkings. Two brushing sequences, as recommended by the manufacturer, were used in this study.

4.5 Evaluation of teat cleaning (II)

Investigators monitored a single milking in autumn 2003. Udder and teat structure, udder hairiness, and teat colour, condition, and cleanliness were evaluated by the same experienced person before the AMU cleaned the teats. During teat cleaning, the technical success of the teat cleaning (TSTC) and behaviour of the cow were evaluated. After teat cleaning, teats that were successfully (technically) cleaned were evaluated for teat cleanliness to assess the effectiveness of teat cleaning (ETC).

4.5.1 Classification of technical success of teat cleaning

TSTC was recorded as:
1) successful (teat straight and residing completely in the cleaning device throughout the cleaning process)
2) partly unsuccessful (teat folded against the udder base or otherwise only partially in the cleaning device, or not in the cleaning device at all for the whole time of cleaning)
3) totally unsuccessful (teat not in the cleaning device or the cleaning process not started by the AMU).

4.5.2 Effectiveness of teat cleaning – visual evaluation

![Figure 2. The scoring system to assess cleanliness of the teat. From left to right: a score of 0 = clean (no visible dirt), 1 = almost clean (approximately <10% of the area dirty), 2 = slightly dirty (10 to 20% of the area dirty), 3 = dirty (20 to 50% of the area dirty), and 4 = extremely dirty (>50% of the area dirty).](image)
4.5.3 Effectiveness of teat cleaning - evaluation of cloths

After teat cleaning, two teats per cow were swabbed with a 6 x 15 cm white rectangular fiber cloth moistened with water. The moistened cloth was wrapped around the teat and stroked once from the teat base to apex. After sampling, cloths were dried at room temperature. Cloths were classified visually into classes 0-4 (Figure 4). Not only the dirty area, but also the thickness of the dirt was taken into account during visual evaluation.

Figure 4. The scoring system used to assess cleanliness of a teat-cleaning cloth, for which score 0 = clean (no visible dirt), 1 = almost clean, 2 = slightly dirty, 3 = dirty, and 4 = extremely dirty.

Figure 5. Assessment of the cleanliness of a teat-cleaning cloth using an optical system. The central circle of this particular cloth was selected for analysis.
Cleanliness of the cloths was also analysed with a Minolta Chroma meter CR-210 colorimeter (Minolta Co Ltd.), which measures reflected light from the cloth: the dirtier the cloth, the less the reflection. Three circular measurements were taken from each cloth (Figure 5), and the smallest value (i.e. the dirtiest circle on the cloth) was selected.

4.6 Milk sampling and determination of indicators of inflammation in milk

4.6.1 Milk sampling (III, IV)

Milk samples (35 ml in study III and 25 ml in study IV) were collected for cow-side EC measurement (IV), SCC and evaluation of milk appearance. Samples were taken before milking, after discarding the first streams of milk. Samples (2 ml) were stored at -20 °C for milk N-acetyl-β-D-glucosaminidase (NAGase) activity analysis (IV). Samples for bacterial analysis were taken using an aseptic technique. Samples for SCC, NAGase activity and bacterial analysis were cooled immediately in the refrigerator and sent to the laboratory.

4.6.2 Milk SCC (III, IV)

Quarter milk SCC was determined using a fluoro-optic method (Fossomatic Milk Analysis, Foss Electric, Hillerød, Denmark) in study III, and using an electronic counter DCC (DeLaval International AB, Sweden) in study IV. CMT scores were determined using the Scandinavian scoring system of Klastrup and Schmidt Madsen (1974) in study III.

4.6.3 NAGase activity (IV)

Milk NAGase activity was measured with a fluorometric method, modified from Mattila (1985). The calibrated milk sample (Mattila, 1985) was replaced with a control milk sample of known 4-MU concentration and NAGase activity was expressed as pmoles of 4-MU/min/μL milk at room temperature, instead of arbitrary units.

4.6.4 Electrical conductivity (IV)

Electrical conductivity was measured with a hand-held meter (Lutron CD-4301, Lutron Electronic Enterprise Co., Taipei, Taiwan).

4.6.5 Bacterial identification (III, IV)

Bacteria were cultured and identified using conventional methods (Hogan et al., 1999).
4.7 Mastitis detection and method manipulation in automatic milking (III)

The mastitis detection system for Group A included on-line quarter-based EC and milk yield measurements with Free-Flow FF4/VC (DeLaval International AB, Sweden). Details of the milk yield detection method were not provided by the manufacturer. An alert based on EC was given for a quarter when the mean EC of the quarter, measured continuously, was ≥ 15% higher than the mean for the two quarters with the lowest values (inter-quarter ratio A, IQRA ≥ 1.15). The threshold value for automatic separation of milk was 7500 mS/cm.

The mastitis detection system for Group B included on-line quarter-based EC and milk colour measurements with MQC® (Lely Industries, Netherlands). An alert based on EC was given for a quarter if EC (measured in indices) of the quarter was ≥ 20% higher than in the quarter with the lowest value (IQRB ≥ 1.20) during two consecutive milkings. In addition, IQRB of the running average of the three last milkings had to exceed 1.20. Automatic separation of milk took place when IQRB was ≥ 1.70. A milk colour sensor (Espada and Vijverberg, 2002) provided the following alerts: colostrum, abnormal milk, mastitic milk, 1st milk (first 100 ml) and milk with blood. Threshold values for automatic separation of milk were not provided by the manufacturer.

Details of the EC detection are presented in Table 3. In addition to the manufacturer’s criteria (M) new test criteria (T) were examined, which were used for all data in study III. Fullfilment of the criteria of an alert differed in lowest EC value that was accepted as a successful measurement by the AMU, IQR that had to be exceeded, and wether to incorporate a requirement of a running average of EC or EC at previous milking to exceed the IQR limit also.

Table 3. Criteria for alerts based on electrical conductivity used for the data from Groups A and B. Manufacturer’s criteria (M1, M2 and M3) and criteria tested (T1, T2, T3 and T4) are shown.

<table>
<thead>
<tr>
<th>EC criteria</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest possible EC³, µS/cm or index</td>
<td>M1¹</td>
<td>M2²</td>
</tr>
<tr>
<td>Inter-quarter ratio, %</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Running average of 3 milkings included</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Previous measurement included</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

¹M1 was used during the study period
²M2 is currently in use
³If Ec is below this threshold, the measurement is not used for inter-quarter ratio calculation
+ included in the criteria
- not included in the criteria
4.8 Evaluation of the performance of AMS to detect mastitis (III)

4.8.1 Sensitivity

1) SE of detecting subclinical mastitis was assessed as the proportion of subclinical quarters receiving an alert at test milking and for 10 preceding milkings. Only cows with an acceptable milking interval from the last milking (≥ 5 h for quarters with SCC ≤ 400 000 cells/ml and ≥ 6 h for quarters ≤ 200 000 cells/ml) before milking were included.
2) SE of detecting quarters with abnormal milk was determined for the day mastitis was recorded and for the 5 preceding days.

4.8.2 Specificity

SP of distinguishing between healthy quarters and those with subclinical mastitis was assessed as the proportion of healthy quarters (i.e. quarters with less than 200 000 cells/ml) receiving no alert at the test milking.

4.8.3 False alert rate, positive and negative predictive value

False alert rate was defined as FP alerts per 1000 cow milkings, and was counted as (100 – SP)*10 (Scherlock et al., 2008). Here, SP was assessed as the proportion of healthy cows (i.e. cows with all quarters having SCC below the chosen threshold) receiving no alerts at the test milking.

Positive predictive value (PPV) was defined as the proportion of TP alerts of all alerts at the test milking. Negative predictive value (NPV) was defined as the proportion of TN alerts of all quarters receiving no alerts at the test milking.

4.8.4 Classification of alerts

1) At test milking: an alert was considered to be a false positive (FP) when SCC was < 200 000 cells/ml.
2) At follow-up period: the number of alerts/quarter and the proportion of true positive (TP) alerts were determined. The minimum requirement for a TP alert was that the quarter had a CMT ≥ 3 the day before or after the alert or during the day of the alert. If more than one alert was received for a particular quarter within a few days, the alert was considered TP if most of the alerts were TPs, otherwise the alert was not determined. If CMT score was always < 3, i.e., < 300 000 cells/ml, (Klastrup & Schmidt Madsen, 1974), the alert was considered to be a FP.
4.9 Induction of experimental mastitis (IV)

The experimental period was 5 d, referred to as d -1, 0, and 1-3. Day -1 served as a control day for the thermal camera adjustment. The left fore udder quarter of all six cows was infused with 5 ml of sodium chloride (NaCl) on d -1, at 07.00. On d 0, the same quarter was infused with 10 µg of E. coli O55:B5 lipopolysaccharide (LPS) (Sigma, Sigma-Aldrich, Inc., MO) diluted in 5 ml of NaCl. The right fore quarter served as an intact control. The experimental protocol is shown in Table 4.

Table 4. Protocol for inducing experimental mastitis in 6 cows with E. coli LPS.

<table>
<thead>
<tr>
<th>Time of the day (h)</th>
<th>Days</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>11</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, B</td>
<td>-1</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Y</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Y</td>
<td>A</td>
</tr>
<tr>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Y</td>
<td>A</td>
</tr>
<tr>
<td>LPS</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Y</td>
<td>A</td>
</tr>
<tr>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<td>A</td>
</tr>
<tr>
<td>A, B</td>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<td>A</td>
<td>Y</td>
<td>A</td>
</tr>
<tr>
<td>A, B</td>
<td>3</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>Y</td>
<td>A</td>
</tr>
</tbody>
</table>

1 A = clinical examination of the cow, milk sampling and thermal imaging of experimental and control quarters
2 B = bacterial sampling of milk
3 NaCl = NaCl infusion of the experimental quarter
4 LPS = Escherichia coli LPS infusion of the experimental quarter

4.10 Thermal camera (IV)

The thermal camera used (IR FlexCam Pro, Infrared solutions, Fluke Company, Everett, WA) operated in the 8 to 14 µm spectral band. The thermal resolution of the camera was 0.09 °C, calibrated from 0 to 100 °C. The camera used micro bolometer detectors and had a 160 by 120 focal plane array. It had an internal recalibration feature, which automatically calibrated the detector to give correct readings for the ambient temperature. The emissivity value was set to 0.98, which was the value used for measuring the temperature of human skin (Jones and Plassmann, 2002).

4.11 Thermal imaging (IV)

Thermal images were taken to monitor changes in the udder skin temperature (TU) of the experimental and control quarters. Images were taken with the handheld thermal camera at every sampling before clinical examination and milk sampling of the udder, approximately 50 cm from the udder. The orientation of the images was from the lateral and medial angles of the quarters. The maximum temperature of the image was not always on the area of the target quarter and therefore the mean temperature within a circle of 40 x 40 pixels above the teat was measured. The circle was drawn by hand with the help of the camera...
software, starting just above the teat and centred according to the teat (Figure 6). This area expressed the smallest normal variation in temperature of the udder (Barth, 2000). An example of the thermal images before and during the inflammation is shown in Figure 6.

Figure 6. Thermal images of the lateral angle of the experimental udder quarter (left fore) a) before and b) during the infection. A1 = 40 X 40 pixel area above the teat, Hot+ = position of the maximum udder skin temperature of the image.

4.12 Statistical analysis

4.12.1 Models used in study I

All statistical analyses were done with the SAS 9.1 (SAS Institute Inc., Cary, NC). LogSCC was tested with a mixed model:

\[
\text{LogSCC} = \text{Period} + \text{Barntype0} + \text{Barntype1} + \text{Barntype1} \times \text{Period} + \text{Barntype1} \times \text{Period} \times \text{Parity} + \text{Month} + \text{Year} + \text{Parity} + \text{ExpDIM} + \text{DIM} + \text{DIM} \times \text{DIM} + \text{Error}
\]

HerdID was included as a random effect and other variables as fixed effects. Period was a year before and after the change. Barntype0 before the change was either tie stall or free stall barn with CM and Barntype1 after the change was free stall barn with either CM or AM. Parity was included as 1st, 2nd, or older. The variables ExpDIM (exponential function of DIM), DIM and DIM*DIM modelled the lactation curves. Month and Year were calendar months and years. The interaction between Period, Barntype1 and Parity tested the effect of different lactations. Model 1 included BovineID as a repeated variable having test date as subject and using the autoregressive structure (AR(1)). Data were unbalanced regarding breed and it was left unaccounted for. Herd size was left unaccounted for because it was covered by the fixed effect of Parity and the random HerdID effect.
The percentage of cows on a farm that had an SCC > 200 000 cells/mL for the first time within a two year period (highSCC) was used to explore the number of new cases of mastitis. HighSCC was calculated based on the cows at risk. To test the dichotomous outcome that a cow was associated with highSCC for the first time, a categorical procedure (PROC GENMOD) was used. BovineID within herd was included as a repeated subject. The outcome was a logit value for the probability of having highSCC.

\[
(2) \quad \text{HighSCC} = \text{Period} + \text{Barntype0} + \text{Barntype1} + \text{Barntype1*Period} + \text{Month} + \text{Year} + \text{Parity} + \text{ExpDIM} + \text{DIM} + \text{DIM*DIM} + \text{Error}
\]

In addition, least squares mean values for milk yield, logSCC and the proportion of highSCC cows were calculated over the months before and after the change to study the temporal effect of the change by substituting Period for Month relative to the change in Models 1 and 2, yielding Models 1a and 2a. The models did not converge for repeated measures of ECM and highSCC, but still produced correct least squares mean values without the repeated statement. These values were employed to show the development of ECM and highSCC after the change in housing or milking system or both.

Treatments for clinical mastitis as cases per 10 000 cow days within herd and period were tested with a mixed model. Treatments were log-transformed before analysis to normalise the distribution. Treatments of the same cow within one week were considered as a single case. Parity was included as 1st or older.

\[
(3) \quad \text{Treatments} = \text{Period} + \text{Parity} + \text{Barntype0} + \text{Barntype1} + \text{Barntype1*Period} + \text{Barntype1*Period*Parity} + \text{Error}
\]

### 4.12.2 Models used in study II and III

All analyses were done using SPSS 11.0. (SPSS Inc., Chicago, IL). Pearson’s $\chi^2$ or Student’s t -test was used to test the independence of different variables between groups and the interdependence of the covariates. Confidence intervals at the 95% level were estimated for cell frequencies by normal approximation of a binomial distribution. The experimental unit was the teat, with the exception of the cow-level characteristics and the cow-level comparison of the groups. In study II factors associated with TSTC were studied with a binary logistic regression model. The model was repeated separately for both groups and accounted for the effect of herd and characteristics of the teats and cows. Herd was included to control for the cluster effect of the herds. The initial model was:

\[
(4) \quad \text{Successful teat cleaning} = \mu + \text{Herd} + \text{Teat position} + \text{Parity} + \text{DIM} + \text{Milking frequency} + \text{Time since last milking} + \text{Cow behaviour} + \text{Teat colour} + \text{Udder and teat structure} + \text{Udder hairiness}
\]

Herd (1 to 9) was characterised as a categorical variable. All other covariates were dichotomous. Teat position was either fore or hind. Statistically significant ($P < 0.05$) variables were included in the final model and selected with the help of the backward selection procedure.
Cow and herd characteristics associated with ETC were studied with an ordinal regression model with a negative log-log link-function. The initial model accounted for the effect of group, herd and characteristics of the teats and cows, and was:

\[
(5) \quad \text{Teat cleanliness after cleaning (0 to 4)} = \mu + \text{Group} + \text{Herd} + \text{Teat position} + \text{Teat cleanliness before cleaning} + \text{Parity} + \text{DIM} + \text{Milking frequency} + \text{Time since last milking} + \text{Teat colour} + \text{Udder and teat structure} + \text{Teat condition} + \text{Udder hairiness} + \text{Group*Teat cleanliness before cleaning (1 to 4)} + \text{Herd*Teat cleanliness before cleaning}.
\]

Statistically significant \((P < 0.05)\) variables were included in the final model.

Data for teat cleanliness after cleaning, gathered using 3 methods (direct visual assessment of the teats and evaluation of cleanliness of the cleaning cloths visually and using the optical method), were compared using correlation analysis.

In study III Student’s t -test was used to evaluate the independence of healthy and subclinical quarters. The relationship between SCC and EC was tested using Spearman’s correlation coefficient.

### 4.12.3 Models used in study IV

All statistical analyses were conducted with the SPSS 13.0 (SPSS Inc., Chicago, IL). The effect of mastitis on the indicators of inflammation in the milk and \(T_U\) were analysed with a mixed model that took repeated measures into account. Fixed factors were the hour (the time from mastitis induction), quarter (experimental or control), and the interaction between the hour and quarter. The cow was considered to be a random effect. No covariates were used. \(T_R\) was analysed with a model equivalent to the model described above, except that only the hour was a fixed effect. Normality of distribution was analysed from the residuals. Homogeneity of variances was evaluated with a scatter plot of residuals and predicted values. Pearson’s correlation coefficient was calculated between \(T_R\) and \(T_U\).
5 RESULTS

5.1 Udder health and mastitis treatments of cows in automatic vs. conventional milking

5.1.1 Average logSCC and proportion of new high SCC cows

Changes in udder health, assessed by mean logSCC of the individual cows and proportion of new high SCC cows, were followed up in dairy herds after transfer from a tie stall or free stall barn with CM to free stall barn with CM or AM (period 0 = before, period 1 = after). The logSCC increased from period 0 to period 1 (P < 0.001) (Table 5). Cows in the tie stalls had lower logSCC than cows in free stall barns during period 0 (P < 0.001). An interaction was established between periods in AM and CM herds; logSCC of AM cows increased from 4.89 ± 0.01 to 4.96 ± 0.02 compared with an increase from 4.87 ± 0.02 to 4.90 ± 0.02 for CM cows (P < 0.001). This corresponds to an increase in cow SCC of approximately 14 000 cells/ml in AM herds compared with 5 000 cells/ml in CM herds. Older cows showed a greater increase in logSCC than younger cows, from 5.00 ± 0.01 to 5.09 ± 0.02 compared with an increase from 4.97 ± 0.02 to 5.02 ± 0.02 for younger cows (P = 0.018). Both AM and CM groups had an increase in logSCC before the change in housing and milking system (Figure 7), but logSCC in AM herds continued to a higher level after the change; the difference between groups became significant at month 4 (P = 0.015) and highly significant at months 10 and 12 after the change (P < 0.001).

Figure 7. Least squares means of logSCC (±SE) (Model 1a) relative to the change from tie stall or free stall barn with conventional milking to a) free stall barn with conventional milking (94 farms; broken line) or b) to free stall barn with automatic milking (88 farms; solid line). * P < 0.05 compared between groups; ***P < 0.001.
A general increase of 0.5% was noted in the proportion of new highSCC cows from period 0 to period 1 ($P = 0.001$) (Table 5), calculated from cows at risk. The proportion of new highSCC cows was higher in the AM group compared with the CM group in period 1 ($P = 0.011$). An interaction existed between periods in AM and CM groups; in AM cows the proportion of new highSCC cows increased from 2.8% to 3.8%, whereas in the CM herds the increase was from 2.0% to 2.2% ($P = 0.006$). Increasing age of the cows was associated with a lower proportion of new highSCC cows (parities 1, 2 and $\geq 3$; 3.6, 2.8 and 1.8, respectively; $P < 0.001$). An apparent adaptation period of 2 to 3 months seemed to occur after the change in milking systems in the AM group (Figure 8). The difference between the two groups after the change was significant, although the statistical model could not indicate which months differed. The proportion of highSCC cows appeared to stabilise close to the original values towards the end of the study.

Figure 8. Least squares means of proportion of new high SCC cows (> 200 000 cells/ml) (Model 2a) relative to the change from tie stall or free stall barn with conventional milking to a) free stall barn with conventional milking (94 farms; broken line) or b) to free stall barn with automatic milking (88 farms; solid line).
Table 5. Least squares means (± SE) and levels of significance (P) of Log SCC, the proportion of cows at risk having SCC > 200 000 for the first time.

<table>
<thead>
<tr>
<th>Product</th>
<th>ExpDM</th>
<th>DIM</th>
<th>DIM×DIM</th>
<th>Per</th>
<th>Per×Per</th>
<th>Year</th>
<th>Per×Year</th>
<th>M</th>
<th>BTL</th>
<th>BTL×BTL</th>
<th>Par</th>
<th>Par×Par</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Note:**
- **ExpDM:** Expected dry matter intake
- **DIM:** Daily intake of milk
- **DIM×DIM:** Interaction term for daily intake of milk
- **Per:** Percentage
- **Per×Per:** Interaction term for percentage
- **Year:** Year
- **Par×Year:** Interaction term for percentage by year
- **BTL:** Birth type
- **BTL×BTL:** Interaction term for birth type
- **Par:** Parity
- **Par×Par:** Interaction term for parity

Levels of significance:
- **ns** (not significant)
- **** (P < 0.05)
- **** (P < 0.01)
- **** (P < 0.001)

**Log SCC:** Log of somatic cell count

**SE:** Standard error

**P:** Level of significance
5.1.2 Mastitis treatments

The number of recorded treatments of clinical mastitis tended to be 0.8/10 000 cow-days higher in period 1 than in period 0 ($P = 0.077$) (Table 5). The interaction between periods in AM and CM groups was significant ($P = 0.05$); in AM herds the number of recorded treatments decreased from period 0 to period 1, from 5.3 to 5.1 treatments per 10 000 cow-days, whereas in CM herds the number increased from 3.0 to 4.6 treatments per 10 000 cow-days. This corresponds to approximately 0.2 treatments less per year in AM and 3 treatments more per year in CM in a 60 cow herd. First parity cows had fewer treatments than older cows (1.0 vs. 18.9 treatments per 10 000 cow-days; $P < 0.001$). In CM herds, all cows were treated more often in period 1, but in AM herds first parity cows had fewer and the older cows more treatments in period 1 than period 0 (parity 1: CM from 1.1 to 1.5 and AM from 0.8 to 0.6 treatments per 10 000 cow-days, older cows: CM from 7.9 to 13.2 and AM from 30.5 to 39.7 treatments per 10 000 cow-days; $P < 0.001$).

5.2 Teat cleaning in automatic milking

5.2.1 Technical success of teat cleaning

Teat cleanings at the quarter level were more successful in Group B than in Group A ($P = 0.012$) (Figure 9). In Group B, 63.0% of the partly unsuccessful and 80.0% of the totally unsuccessful cleanings failed in both brushing sequences. Within Group A, the proportion of successful teat cleanings significantly differed between the herds (Wald test value 40.9 with 4 degrees of freedom, $P < 0.001$) (Figure 9). Reasons for the failures are given in Table 6. According to the odds ratios of the logistic regression model (Table 7), each of the factors associated with TSTC reduced chances of a successful teat cleaning to half or less. The model was able to explain on average 97.3% of the successful teat cleanings, but only 7.5% (Group B) or 29.2% (Group A) of the unsuccessful teat cleanings.

Figure 9. Technical success of teat cleaning for cows and quarters in Groups A and B in study II (with minimum and maximum for different herds).
Table 6. Reasons for partly and totally unsuccessful teat cleanings in Group A (cleaning cup) and Group B (rotating brushes).

<table>
<thead>
<tr>
<th>Observed reason for failure</th>
<th>Partly unsuccessful</th>
<th>Totally unsuccessful</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>65 %</td>
<td>54 %</td>
</tr>
<tr>
<td>Behaviour of the cow</td>
<td>12 %</td>
<td>21 %</td>
</tr>
<tr>
<td>Udder structure</td>
<td>14 %</td>
<td>25 %</td>
</tr>
<tr>
<td>Udder hair</td>
<td>9 %</td>
<td></td>
</tr>
<tr>
<td>Device failure</td>
<td></td>
<td>52 %</td>
</tr>
</tbody>
</table>

Table 7. Factors associated with technical success of teat cleaning (TSTC) according to a logistic regression model.

<table>
<thead>
<tr>
<th>Factors associated with TSTC (reference)</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR^1 95% CI</td>
<td>% Successful teat cleanings if reference present</td>
</tr>
<tr>
<td>Teat location (hind)</td>
<td>0.47** 0.29–0.74</td>
<td>50</td>
</tr>
<tr>
<td>Behaviour (restless/not centered at the stall)</td>
<td>0.27** 0.12–0.63</td>
<td>50</td>
</tr>
<tr>
<td>DIM (until d 30 postpartum)</td>
<td>0.25*** 0.14–0.45</td>
<td>50</td>
</tr>
<tr>
<td>Teat structure (abnormal)</td>
<td>Not associated 71</td>
<td>10.4 (2.5)</td>
</tr>
<tr>
<td>Teat colour (&gt;50% black)</td>
<td>0.41** 0.23–0.73</td>
<td>70</td>
</tr>
</tbody>
</table>

^1OR = Odds ratio of the factor  
** ** P < 0.001, ** P < 0.01  
^a,b proportions with different superscript letters are significantly different between groups (P < 0.05)
5.2.2 Effectiveness of teat cleaning

5.2.2.1. Visual evaluation of the teats

After cleaning, 33.1% of the teats in Group A (cleaned with a cup) and 37.1% of the teats in Group B (cleaned with brushes) were totally clean \((P = 0.168)\). If a dichotomous scale was used, 84.5% and 80.6% of the teats were considered clean \((P = 0.094)\). Four teats were classified as dirtier after cleaning than before cleaning.

Figures 10 and 11 show that in Group A, a larger proportion of the extremely dirty teats were clean or almost clean after cleaning \((P = 0.002)\) than in Group B. For originally dirty teats the cleaning cup was more efficient than the brushes, with a higher percentage of teats becoming clean or almost clean during the cleaning process (80% vs. 73%) \((P = 0.024)\). According to the ordinal regression model, ETC was associated with the group (Wald test value of Group A was 8.8, \(P = 0.003\)), and there was a significant interaction between the group and teat cleanliness before cleaning (Wald test value for Group A = 30.0, \(P < 0.001\); and Group B = 60.3, \(P < 0.001\)). Variation of ETC between the herds was slightly larger than between the groups. The interaction term ‘herd \(\times\) teat cleanliness before cleaning’ was significantly associated with ETC in only one herd. Cleanliness of the teat after cleaning varied mainly according to cleanliness before cleaning (Wald test value of change of one cleanliness category 56.7, \(P < 0.001\)).

Figure 10. Teat cleanliness after teat cleaning with a cleaning cup for different categories of teat cleanliness before cleaning (472 teats).
Cleaning of the orifices of the teats was less efficient than cleaning the teat barrel or apex. In Group B, cleaning teat orifices was inferior compared with in Group A ($P = 0.039$; Figure 12). Teat condition was associated with ETC (Wald test value $= 7.5$, $P = 0.006$). Of the healthy teat ends in Groups A and B, 50.4% were cleaned, whereas of the teats having a callosity ring, only 34.3% were cleaned ($P = 0.005$). Bedding material on the teats was
almost completely removed (Figure 12). In Group A, bedding material was peat (2 herds) or sawdust (2 herds); for one herd, no bedding was used. In Group B, sawdust was used for 3 herds and straw for one herd.

5.2.2.2 Evaluation of cloths

Visual evaluation of cloths revealed no statistical differences between Groups A and B in the cleanliness of the cloths after cleaning (Table 8). Compared with the visual evaluation of the teats, showing that teats were mostly “almost clean” after teat cleaning, cloths taken after cleaning of the teats were mostly classified as “slightly dirty”, even though the two scales cannot be directly compared. Correlation between the results of visual evaluation of teat cleanliness and visual evaluation of the cloths was 0.42 (P < 0.01), and between visual evaluation of teat cleanliness and optical evaluation of the cloth -0.44 (P < 0.01) and between visual and optical evaluation of the cloths -0.73 (P < 0.01).

Table 8. Comparison of three methods of teat cleanliness evaluation after teat cleaning: visual evaluation of teat cleanliness (Visual – teat), visual evaluation of the cloths used to swab the teats (Visual – cloth) and measurement of cloth dirtiness using an optical system (Optical – cloth) (CI = confidence interval).

<table>
<thead>
<tr>
<th>Visual - teat</th>
<th>Number of teats</th>
<th>Visual - cloth mean</th>
<th>Visual - cloth median</th>
<th>Optical - cloth mean</th>
<th>Optical - cloth CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>198</td>
<td>1.7</td>
<td>2</td>
<td>86.4</td>
<td>0.01</td>
</tr>
<tr>
<td>1</td>
<td>303</td>
<td>2.0</td>
<td>2</td>
<td>85.1</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>2.5</td>
<td>2</td>
<td>83.3</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>2.7</td>
<td>3</td>
<td>81.6</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>3.3</td>
<td>4</td>
<td>80.5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

5.3 Detection of mastitis in automatic milking

5.3.1 Subclinical mastitis

Diagnostic efficiency of EC measurement with different EC criteria is shown in Table 9. Most FP alerts in Group A originated from quarters of healthy udders (udders with no quarters with high SCCs). Correlation between SCC and EC was ≤ 0.4 (P < 0.01), depending on the quarter. Mean EC and IQR of all quarters with or without subclinical mastitis are shown in Table 10.

If the cut-off for subclinical mastitis had been 100 000 cells/ml, 35 and 70 more cases of mastitis would have been found in Groups A and B, with 2 and 0 more alerts during the test milking. SE for the test milking would have been reduced to approximately 14.1% and 3.3%, respectively. SP would have increased to 98.8 and number of FP decreased to 4 out of 17 in Group A, increasing PPV to 76.5%. Negative predictive value (NPV) would have increased in both groups (to 80.9 and 68.1%).
Table 9. Detection of quarters with subclinical mastitis based on electrical conductivity of milk from cows in Groups A and B using different AMS brands. Sensitivity (SE), specificity (SP), false positive (FP) of positive alerts (P), positive predictive value (PPV), negative predictive value (NPV) and false alert rate per 1000 cow milkings (FAR).

<table>
<thead>
<tr>
<th>Cell cut-off Quarters</th>
<th>Alg.</th>
<th>SE %</th>
<th>SE10 %</th>
<th>SP %</th>
<th>FP/P</th>
<th>PPV %</th>
<th>NPV %</th>
<th>FAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A 430 quarters</td>
<td>M1</td>
<td>18</td>
<td>32</td>
<td>98.4</td>
<td>6/17</td>
<td>65</td>
<td>89</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>16</td>
<td>43</td>
<td>99.2</td>
<td>3/12</td>
<td>75</td>
<td>89</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>9</td>
<td></td>
<td>99.5</td>
<td>2/7</td>
<td>71</td>
<td>88</td>
<td>11</td>
</tr>
<tr>
<td>Group B 655 quarters</td>
<td>M1</td>
<td>32</td>
<td>50</td>
<td>97.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>29</td>
<td>50</td>
<td>99.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>14</td>
<td></td>
<td>99.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>5</td>
<td>13</td>
<td>100.0</td>
<td>0/7</td>
<td>100</td>
<td>79</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>8</td>
<td>20</td>
<td>99.8</td>
<td>1/13</td>
<td>92</td>
<td>79</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>13</td>
<td></td>
<td>99.4</td>
<td>3/22</td>
<td>86</td>
<td>80</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>9</td>
<td>20</td>
<td>99.8</td>
<td>1/26</td>
<td>92</td>
<td>79</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>13</td>
<td></td>
<td>99.3</td>
<td>1/26</td>
<td>92</td>
<td>79</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>19</td>
<td></td>
<td>98.5</td>
<td>1/26</td>
<td>92</td>
<td>79</td>
<td>13</td>
</tr>
</tbody>
</table>

1 For different EC criteria; see Table 5. M1 (Manufacturer 1), T1 (Test 1) and T2 (Test 2) represent different EC criteria in Group A, and M3 (Manufacturer 3), T3 (Test 3), and T4 (Test 4) represent different EC criteria in Group B.

2 SE calculated from 10 milkings prior to the test milking.

Table 10. Mean EC and IQR of healthy quarters and quarters with subclinical or clinical mastitis in Groups A and B using different AMS brands.

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean EC µS/cm (Range)</td>
<td>Mean IQRA</td>
</tr>
<tr>
<td>Healthy</td>
<td>4729&lt;sup&gt;a&lt;/sup&gt; (3484-7169)</td>
</tr>
<tr>
<td>High SCC</td>
<td>5059&lt;sup&gt;b&lt;/sup&gt; (3752-7437)</td>
</tr>
<tr>
<td>Clinical</td>
<td>6360&lt;sup&gt;b&lt;/sup&gt; (4623-7102)</td>
</tr>
</tbody>
</table>

<sup>**</sup><sup>** P < 0.001</sup>
In Group A (total of 430 quarters) 30 quarters had IMI (of them 21 had SCC ≥ 200 000 cells/ml) at the test milking; only 6 quarters had an alert, all with SCC ≥ 200 000 cells/ml. In Group B (total of 655 quarters), 72 quarters had IMI (of them 59 had SCC ≥ 200 000 cells/ml) at the test milking; only 3 quarters had an EC alert, all with SCC ≥ 200 000 cells/ml. If the definition of subclinical mastitis had been IMI, the number of cases would have dropped by 50%, but SE would have remained approximately the same. Using the definition IMI + ≥ 200 000 cells/ml, the number of cases would have dropped by 60% and SE increased by 10% in Group A.

SE of milk colour analysis in detecting quarters with a high SCC ranged from 0.7% to 4.3%, depending on the SCC-limit. SP was always 99.8%. One of the two alerts during the test milking was a FP and came from a cow with another quarter with > 1 000 000 cells/ml milk. Colour index values between quarters that were healthy or had a high SCC did not differ ($P > 0.05$).

### 5.3.2 Clinical mastitis

Table 11. Detection of quarters with clinical mastitis or milk with blood, based on milk EC (Groups A and B) or milk colour (Group B) measurement.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
<td>EC/colour $^2$ in total</td>
</tr>
<tr>
<td><strong>Clinical mastitis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. quarters/cows/herds</td>
<td>7/6/3</td>
<td>17/17/4</td>
</tr>
<tr>
<td>Quarters with alerts at the day of diagnosis</td>
<td>4</td>
<td>10/8 = 14</td>
</tr>
<tr>
<td>Quarters with alerts during 5 preceding days</td>
<td>5</td>
<td>10/9 = 13</td>
</tr>
<tr>
<td>Quarters with alerts in total (6 days)</td>
<td>5</td>
<td>11/11 = 16</td>
</tr>
<tr>
<td><strong>Milk with blood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. quarters/cows/herds</td>
<td>3/3/3</td>
<td>5/5/2</td>
</tr>
<tr>
<td>Quarters with alerts at the day of diagnosis</td>
<td>1</td>
<td>0/3 = 3</td>
</tr>
<tr>
<td>Quarters with alerts during 5 preceding days</td>
<td>1</td>
<td>1/5 = 5</td>
</tr>
<tr>
<td>Quarters with alerts in total (6 days)</td>
<td>1</td>
<td>1/5 = 5</td>
</tr>
</tbody>
</table>

$^1$ One cow had two different quarters with clinical mastitis two months apart

$^2$ Colour alerts were defined: abnormal milk, mastitic milk, 1st milk, and colostrum milk.

$^3$ Not included in clinical mastitis category

Detection of quarters with clinical mastitis or with milk containing blood in Group A and B is shown in Table 11. Milk was automatically separated in only 4 of 7 cases of clinical mastitis in Group A and 3 (EC) or 2 (colour) of 17 cases in Group B. In Group B, with EC test criteria T3 and T4, two more quarters with clinical mastitis were detected on the day of the diagnosis. One (T3) and two (T4) more quarters with milk containing blood had alerts both before and during the day of diagnosis. For 4 cows, EC values for several time points were not given by the AMS in Groups A and B, and two cows lacked some colour.
values. Only one quarter with bloody milk had a „bloody milk’ alert (and diversion) one day after the diagnosis. One of the 4 cows having colostrum during the test day did not have any colour alerts before or during the test day. Mean EC and IQR of all quarters with clinical mastitis are shown in Table 10.

5.3.3 Reliability of the alerts

Table 12 shows the details of the follow-up period with proportion of EC, milk yield and milk colour alerts of cows or milkings. Tables 13 and 14 show the proportions of cows that received EC alerts with test EC criteria in different quarters in Groups A and B. Cows with milk yield or colour alerts in different quarters are also presented. The tables include the mean number of alerts/quarter and the proportion of true positive alerts.

Table 12. Alerts for Groups A and B during the follow-up period. SD = standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of follow-up days (SD)</td>
<td>21 (8)</td>
<td>27 (7)</td>
</tr>
<tr>
<td>Cows with alerts based on</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC criteria M1(^1) or M3</td>
<td>58 %</td>
<td>27 %</td>
</tr>
<tr>
<td>EC criteria T2 or T4</td>
<td>29 %</td>
<td>41 %</td>
</tr>
<tr>
<td>Milk yield (Group A) or colour (Group B)</td>
<td>84 %</td>
<td>12 %</td>
</tr>
<tr>
<td>Cows with alerts in total milkings during follow-up with alerts(^2)</td>
<td>92 %</td>
<td>43 %</td>
</tr>
<tr>
<td>19 %</td>
<td>16 %</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) M1 (Manufacturer 1) and T2 (Test 2) represent different EC criteria in Group A, and M3 (Manufacturer 3) and T4 (Test 4) represent different EC criteria in Group B.

\(^2\) Only from those cows that had alerts.

Table 13. Electrical conductivity and milk yield (MY) alerts of cows and different quarters, mean number of alerts and the proportion of true-positive (TP) alerts in Group A (113 quarters each) during the follow-up period.

<table>
<thead>
<tr>
<th>Quarter</th>
<th>Cows with alerts % Number of alerts per cow</th>
<th>TP per cow %(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1(^1)</td>
<td>M2</td>
</tr>
<tr>
<td>LF</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>RF</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>LR</td>
<td>37</td>
<td>41</td>
</tr>
<tr>
<td>RR</td>
<td>42</td>
<td>44</td>
</tr>
</tbody>
</table>

\(^1\) M1 (Manufacturer 2003), M2 (Manufacturer current), T1 (Test 1), and T2 (Test 2) represent different EC criteria.

\(^2\) It was possible to classify about half of the alerts.
Table 14. Electrical conductivity and milk colour (MC) alerts of cows and different quarters, mean number of alerts and the proportion of true-positive (TP) alerts in Group B (209 quarters) during the follow-up period.

<table>
<thead>
<tr>
<th>Quarter</th>
<th>M3 1</th>
<th>T3</th>
<th>T4</th>
<th>MC</th>
<th>M3 1</th>
<th>T3</th>
<th>T4</th>
<th>MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>9</td>
<td>10</td>
<td>13</td>
<td>12</td>
<td>81</td>
<td>78</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>16</td>
<td>19</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>10</td>
<td>83</td>
<td>67</td>
<td>70</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>10</td>
<td>12</td>
<td>18</td>
<td>14</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>12</td>
<td>14</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>9</td>
<td>9</td>
<td>13</td>
<td>12</td>
<td>88</td>
<td>88</td>
<td>73</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>16</td>
<td>18</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 M3 (Manufacturer), T3 (Test 3), and T4 (Test 4) represent different EC criteria.  
2 It was possible to classify about half of the alerts.

5.3.4 Distant detection of clinical mastitis using a thermal camera

5.3.4.1 Clinical signs and indicators of inflammation in milk

Experimental mastitis was induced by LPS to test the thermal camera as a means of automatic mastitis detection. All cows developed mild to moderate systemic and local signs, with changes in the milk appearance within 2 h post challenge (PC). Local signs in the udder were severe in 5 cows on the day of the challenge. Three cows showed severe changes in the milk at some samplings. Figure 13 shows \( T_R, T_U, \) milk SCC, NAGase activity and EC for the experimental quarters during the experimental period. \( T_R \) of all cows increased up to or \( >39.2 \, ^\circ C \) in 4 h (4 cows) or 6 h (2 cows) PC. \( T_R \) peaked in all cows 6 h PC.

Systemic signs were recorded until 8 to 10 h PC. All quarters but one were visibly swollen until the end of the experimental period. Milk appearance did not normalise during the entire experimental period. No local signs or changes in the milk appearance or indicators of inflammation in milk were detected in the control quarters (data not shown).
Mean $T_U$ of the experimental and control quarters increased 4 h PC (Figure 13; $P < 0.01$). $T_U$ of any cow did not increase before $T_R$ increased. Correlation between $T_R$ and mean $T_U$ of the lateral angle of the drawn circle was 0.92 ($P < 0.001$) (Figure 14), and correlation between $T_R$ and maximum $T_U$ of the lateral angle of the image 0.98 ($P < 0.001$). The maximum $T_U$ of the image and the mean $T_U$ within the drawn circle increased in parallel for both angles of the experimental and control quarters. At 6 h PC, 5 of the 6 cows showed a clearly increased mean $T_U$ within the drawn circle ($> 1 ^\circ C$). The increase was not recorded for the sixth cow, which only showed a mild increase in $T_R$ (maximum 39.2 $^\circ C$). At 6 h PC, all cows showed a clearly increased maximum $T_U$ of the image (from 1 to 3$^\circ C$). Maximum $T_U$ of the image was approximately 0.6 $^\circ C$ higher at the lateral angles than at the medial angles (data not shown). In most images the point of maximum temperature of the image was at the bend of the hind leg for the lateral angle and at the groove between the right and the left quarters for the medial angle. Images taken from the lateral angle of the experimental quarters indicated higher temperatures than those taken from the control quarters. In contrast, images from the medial angle of the experimental quarter indicated lower temperatures than those taken from the control quarter ($P < 0.01$). The $T_U$ rapidly returned to normal levels, in parallel with or slightly later than $T_R$.

Figure 13. Rectal temperature, maximum udder skin temperature of the image and udder skin temperature of the 40 x 40 pixel area above the teat of the lateral angle of the experimental quarters, mean milk lnSCC, mean NAGase activity and EC of 6 cows throughout the experimental period, ± SE. E. coli LPS was infused at time point 0. Different letters indicate statistically significant differences between mean temperatures ($P < 0.05$) of different sampling times. * indicates that $P < 0.05$ compared with the control quarter (data not shown).

![Figure 13](image_url)

Figure 14. Correlation between rectal temperature of the cow and the mean of the udder skin temperature at lateral angle of the 40 x 40 pixel area above the teat of the experimental udder quarters of six cows throughout the experimental period.

![Figure 14](image_url)


6 DISCUSSION

6.1 Udder health in automatic milking

6.1.1 Effect of change of barn type, milking system or both on udder health

The AM group comprised herds that had undergone a change in their milking system, and one third of them also in their barn type. The CM group comprised herds that had changed barn and milking system, although milking remained conventional, i.e. it was performed by a milker. Cows were younger after the change and the herds were larger. In both groups, udder health deteriorated slightly after the change, but to a lesser extent in CM.

Our results support those of Bielfeldt et al. (2004), who found, based on data from > 500 farms, changing from tie stalls to free stall barn resulted in an increase in cow SCC. They suggested that cows in tie stalls were more adequately maintained, especially if they had access to pasture. Hultgren (2002) on the other hand, studying 196 Swedish farms, recorded no changes in the herd prevalence of high SCC cows after changing from tie stalls to free stall or straw yard systems, but the frequency of veterinary-treated clinical mastitis and teat injuries was lower for cows in the free stall barn systems with CM, taking parity into account. The effect lasted for longer than 18 months. The combination of improved housing and management factors were suggested as explanations for the lower incidence of teat injuries and less clinical mastitis after the change. It must be kept in mind that the number of recorded treatments of clinical mastitis does not necessarily reflect the true incidence of clinical mastitis, but rather the mastitis treatment strategies on the farm (Nyman et al., 2007).

In our study the average cow composite milk SCC per herd was higher, after a change from CM to AM. The similar observation of Kruip et al. (2002) was assumed to result from the irregularity of milking. Short intervals leave less time for the teats to recover from milking, which could affect teat condition, and long intervals provide bacteria time to colonise the teat. In our study, what appeared to be a small increase in cow SCC after introduction of AM might indeed be an indication of subclinical mastitis in one quarter of a preliminary healthy cow. LogSCC continued to be higher throughout the first year after the change in our study. Also in Mulder et al. (2004), test-day SCC of first parity cows from more than 250 farms increased after introduction of AM, and the increase lasted for 2 years after the introduction. According to Danish data from 69 farms, logSCC and the proportion of new high SCC cows increased after the introduction of AM (Rasmussen et al., 2001), but decreased after the adaptation period of a few months, although the proportion of new high SCC cows among cows at risk was higher throughout the first year after the introduction. In our study, an adaptation period was also seen in AM herds, but the proportion of new high SCC cows seemed to decrease nearly to the level before the change. In Rasmussen et al. (2001), the increase in the proportion of new high SCC cows over the first year after the introduction was 1.8%, which is close to our results of 1.0% in the AM herds. Milk SCC of the cows also fluctuated more from low to high, indicating
more new infections (Rasmussen et al., 2001). A recent study of Rasmussen (2006b) on 478 AMS farms supported the earlier results; the increase in the share of new high SCC cows among cows at risk was still detectable after 4 years with AM. The increase did not depend on the year of introduction or on the AMS brand. The authors speculated that the problem was partly related to well-known problems in mastitis detection in AM (Rasmussen et al., 2001).

In our study, the number of treatments increased in young and old cows in CM herds and in older cows in AM herds, probably because of impaired udder health of these cows, reaching a comparative level between the groups. It seems that in spite of the herds being larger after the change to AM, treatments were still used to the same extent as previously. In Hultgren’s study (2002), cited earlier, the trend was opposite as the number of treatments decreased after changing to large free stall barn barns with CM. Changing to more intensive farming may leave less time for individual cows and their treatments. Consequently, focus may be put on therapy at drying-off or drying-off an individual quarter in the middle of lactation. The study of Pedersen and Bennedsgaard (2006) supports this, as they found that the proportion of cows with a blind quarter increased from 7% to 12.5% during the first 6 months after changing to AM. On the other hand, supporting our results of older cows, Bennedsgaard et al. (2004) reported an increase in antibiotic treatments for mastitis in 20 farms after the introduction of AM (0.4 vs. 0.6 treatments/cow year). Some of the farmers with an AM herd increased treatment frequency because they trusted the alerts from the automatic mastitis detection system, which is inadvisable. The possible increase in the use of antimicrobials in AM is an unwanted consequence that merits attention.

Poelarends et al. (2004) showed that logSCC increased after the introduction of AM for 2nd and 3rd parity cows (but not for 1st parity cows) during early lactation. Also in our study, average logSCC was higher in the older cows and mean milk logSCC increased in the older cows to a greater extent than in young cows. This is not surprising, because according to most studies first parity cows have better udder health (lower cow SCC and lower incidence of treatments of clinical mastitis) than older cows (Dohoo and Martin, 1984; Valde et al., 2004; Steeneveld et al., 2008; Persson Waller et al., 2009), except for the first week of lactation (Barkema et al., 1998; Valde et al., 2004). The number of treatments of young cows slightly decreased after the change to AM, but increased in the old cows, even though the proportion of cows with new highSCC increased more among the young cows. Less time spent per cow in intensive farming applies to both young and old cows, but the farmers may be more familiar with “old” cows, i.e. cows that were already in the old barn, recognizing mastitis in them more easily than in the first-calvers. This remains speculation however. The reasons behind the increased proportion of cows with new highSCC among younger compared with older cows remain open. Perhaps the old cows at risk (the healthy cows) that have survived without mastitis have enhanced immune defence and are able to resist mastitis even during the changes in housing. Similar reasoning was done in Valde et al. (2004), where first parity cows had increased incidence of mastitis treatments in early lactation, compared with the older cows. They assumed, that because young cows with mastitis would probably be culled, the remaining cows at second lactation would have higher resistance for mastitis.
Our results indicated that udder health was negatively influenced by the change, but the effect depended more on the change in the milking system from CM to AM than on the change from a tie stall to a free stall barn. One confounding factor is that CM herds on average comprised fewer cows in milk (33) compared with the cows in milk in AM herds (44) after the change, which may have affected the results. However, the impact of the change was small, and partly transient, taking into account that studies were conducted only during the first year after the change.

Although housing may have generally improved following the change, and improved udder health was expected, but not observed. In some farms with a new barn, a sudden need for new cows arises, to enable the farm to operate at full capacity. The additional cows might not be of the best quality, and could, for example, carry subclinical infections. In some barns AMS or a milking parlour may be installed in an existing barn, which is not optimal for loose-housing, and results in problems for cow traffic, which in turn affects milking frequency in AM. Managing the cows under new conditions requires new farmer skills and the situation can be very challenging. In this study, deterioration of udder health was noted already before the change in milking system, as reported by Poelarends et al. (2004). The construction period causes extra stress for animals and people, new cows can disturb hierarchy in the herd, and learning new methods and solving emerging technical problems associated with the new system takes time.

6.1.2 Comparison of udder health in automatic and conventional milking

The average difference in udder health between the AM and CM groups after the change in housing and milking systems was significant for new highSCC cows, and in months 4, 10 and 12 after the change, also for logSCC. A higher test-day SCC in first parity cows on over 200 AM farms was established compared with first parity cows on over 200 CM farms (Mulder et al., 2004). Previous studies comparing AM and CM with similar barn environment and under similar management identified no differences in udder health of the cows (Berglund et al., 2002 (cow SCC); Hamann and Reinecke, 2002; Wirtz et al., 2004; Lopez-Benavides et al., 2006 (subclinical mastitis); Abeni et al., 2008). Occasional studies reported better udder health associated with AM (Berglund et al, 2002 (quarter SCC); Shoshani and Chaffer, 2002; Lopez-Benavides et al., 2006 (clinical mastitis)), but those studies were done only in one or two barns with two herds. It seems that with good management udders can remain healthy regardless of the milking system used.

We did not establish a definite difference in logSCC of cows after changes in housing and milking systems, and therefore we suggest that the observed differences between large farms with CM and AM in BMSCC in Finland depend mostly on decreased separation of high SCC milk using AM compared with CM. In AM, milk is not separated from the mastitic quarter only, but all milk of the cow is separated. This, with the reduced possibility for detecting high SCC cows, may lead to decreased separation of milk with high SCC and an increase in the BMSCC.
6.2 Teat cleaning in automatic milking

6.2.1 Technical success of teat cleaning

In the present study, TSTC was unsatisfactory for more than one-third of the cows tested, which agrees with the results of Jago et al. (2006) on teat preparation by brushing. In some studies, cleaning with a cup or teat brushing was reported to fail in 10% to 20% of the cases at cow level (Hvaale et al., 2002; Kaihilahti et al., 2007). In our study, herd was the most important factor affecting the technical success of the teat cleaning when a cleaning cup was used, and the variation between herds was large. No differences between groups were found at the cow level, but teat cleanings at the teat level were more successful for teats cleaned with brushes compared with those cleaned using a cleaning cup. Comparison of the groups has to be made with caution because of the differences between cows and teat characteristics in the groups and herds. For example, cows with teats cleaned with a cup (Group A) had more black teats and hairy udders, which could interfere with TSTC for this system, locating the teats before cleaning by laser. On the other hand, cows cleaned with brushes (Group B) were younger and had longer MIs and twice as many restless cows per herd during the cleaning as in Group A, possibly affecting the results for this system, which does not locate the teats before cleaning.

Causes of most of the unsuccessful teat cleanings remained undetermined. The model better explained the successful teat cleanings, with absence of the chosen variables for the model, than the failed ones. Causes of the unexplained, unsuccessful cleanings are probably technical, as reported by Kaihilahti et al. (2007). There is no evidence to suggest that there were incorrect teat coordinates caused by programming errors or by changes in the udder structure after programming. Of the known causes, an undefined device failure for all teats of 6 cows in one herd in the cup cleaning group and restless behaviour in the brushing group, were associated with most of the totally unsuccessful teat cleanings, whereas abnormal udder and teat structure was associated with most of the partly unsuccessful teat cleanings. Most of the unsuccessful brushings were unsuccessful during both rounds, as also reported by Hvaale et al. (2002). The potential benefit of more than one brushing would be a higher ETC and a more efficient milk ejection reflex in some cows. Tangorra et al. (2004) reported that one brushing sequence resulted in lower numbers of bacteria in milk than two (statistical significance was not tested). This is confusing, and they suggested that brushing might favour redistribution of organic matter on the teat surface.

Restlessness of the cow was associated with a lower TSTC in both groups. In cup cleaning, where 10% of the unsuccessful teat cleanings failed due to restlessness, cows can move after localisation of the teat, teats can slip away from the cup, or the cow can kick the cleaning cup off the teat. As an example, Jago et al. (2006) reported 0.1 kicks/brushing of the udder. For teat brushing, where 20% to 50% of the unsuccessful teat cleanings were linked to restless cows or cows standing so that the system could not function properly, cows are expected to stand in the same position as during the previous milking, and remain still. When working with Group B herds, investigators stood on the both sides of the milking stall to locate the cows in the middle of the stall. In general, the cows were calm when observed. More failures in TSTC were found during early lactation,
which may be related to restless behaviour of some cows after calving (van Reenen et al., 2002), udder oedema, or a changing udder shape. Resistance to mastitis is at its lowest level during early lactation (Pyörälä, 2008), and milking hygiene is of utmost importance at that time.

Abnormal udder and teat structure caused some failures in teat cleaning in both groups, although it was significantly associated with TSTC only in teat brushing. The cup cleaning system might be better adapted to abnormal udder structure, but more data should be studied to confirm that assumption. Problems with TSTC were more associated with hind teats than with fore teats. Hind teats close to each other, very thick teats and oblique position of the teat represented the most serious problems in both groups. This supports the results of Miller et al. (1995), who reported that an abnormal distance between hind teats and between fore teats resulted in greatest problems in cluster attachment during AM. Hind quarters were more susceptible to IMI caused by gram- bacteria (environmental bacteria) than fore quarters, possibly because hind quarters were dirtier than fore quarters (Steeneveld et al., 2008) or because of wider diameter of the teat canal in hind teats (Bramley et al., 1981). In our study, it was observed that thick teats caused the cleaning device to attach only to the end of the teat. Black teat pigmentation was associated with unsuccessful teat cleanings during cup cleaning of the teats; this characteristic may cause problems with the attachment of the teat cups. In one herd, the teats were sometimes folded against the udder base with the cleaning cup because the lasers were directed towards the bedding stuck to the long udder hair when attaching the teat cleaning cup. Parity, milking frequency, and time since last milking were not related to the TSTC.

6.2.2 Effectiveness of teat cleaning

The present study clearly showed that there are deficiencies in the ETC of dirty teats in AM. Evaluation of teat cleanliness in this study was visual and, as such, subjective (Knappstein et al., 2002). Nevertheless, our data should be consistent because a single person evaluated all teats. Teat cleanliness after cleaning was also evaluated by wiping the teats with a cloth and evaluating the cleanliness of the cloths. A reasonable correlation between the two methods was established, although cloths were usually more often gauged to be towards the “dirty” end of the cleanliness scale. A similar wiping method was suggested by WestfaliaSurge (Teat cleanliness scorecard 2005, WestfaliaSurge, Inc.).

According to Knappstein et al. (2004), using visual evaluation of the cleanliness of the teats, 69% of them were clean after teat cleaning, which is considerably higher compared with approximately 35% recorded in our study. However, in their study, more than 25% of the teats per brand were already visually clean before teat cleaning, compared with 3-6% in our study. Whether the difference was due to a systematic difference between the evaluation methods, or there was a true difference in cow cleanliness on the farms is not known. Requirements of the ISO-standard (ISO/DIS 20966, 2007) for automatic milking installations were not met in our study herds, particularly as regards teats with more than 10% of the teat area covered with dirt before cleaning.

Teats are cleaned manually in CM, and the effectiveness depends on the method used and the carefulness of the milker. After proper manual teat cleaning, no visible dirt should
remain on any part of the teat (score of 0 in our study). In that respect, our results suggest
that teat cleaning in AM is inferior to well conducted manual teat preparation. This agrees
with the results from some experimental studies in which the initial contamination was
standardised and for which teat cleaning in AM was less effective than manual cleaning
(Schuiling, 1992; Knappstein et al., 2004). In one experiment, measuring spore content of
milk after artificial contamination of the teats furnished contradictory findings; a cleaning
cup was more efficient than manual cleaning with fresh moist tissue paper (Melin et al.,
2002). However, in that study manual preparation and foremilking the whole udder took
only 20 to 24 s/cow, and artificial contamination of the teats may not equate with normal
dirtiness of teats. In field studies with bacteriological and visual evaluation of cleanliness
of the teats, teat cleaning in AM was shown to be less effective than manual cleaning
(Knappstein et al., 2004; Tangorra et al., 2004; Bade et al., 2008). Manual preparation of
the teats reduced the number of total and viable bacteria to a greater extent, and the total
bacterial count on the teat swabs after teat cleaning was lower compared with teat
preparation in AM (Bade et al., 2008). In one study AM teat cleaning was reported to be
similar in effect to manual cleaning (Ten Hag and Leslie, 2002).

Teat cleanliness before cleaning influenced ETC most, supporting the results of
Knappstein et al. (2004). Almost clean or slightly dirty teats were cleaned well, but for
dirty and particularly for extremely dirty teats, the results were poor - about 45% of them
remained dirty. Nearly all teats were visually cleaner or at least no dirtier after cleaning. In
the study of Knappstein et al. (2004), bacterial counts on the teats in some herds increased
during cleaning, in particular if contamination of the teats before cleaning was low and the
teat-cleaning device did not work well or was not itself clean. In the study of Silk et al.
(2003), clipping the udder hair did not affect bacterial load on the teats or the incidence of
IMI, despite the fact that manual teat preparation was carried out only with a dry cloth.
This was also our finding by visual evaluation, suggesting that removal of the udder hair is
more important for general hygiene and for TSTC, as long as the hair does not reach the
teats or contain much manure.

Our finding that there were more differences between the herds than between the teat-
cleaning methods for ETC agrees with the results of Knappstein et al. (2004). Our data
showed, however, that teat cleaning was more effective with a cleaning cup than with
brushes, especially for extremely dirty teats. In contrast, the study of Knappstein et al.
(2004), which was based on bacterial counts on the teat skin before and after teat cleaning,
indicated that extremely dirty teats were cleaned more effectively with brushes, whereas
slightly soiled teats were cleaned more effectively with a cleaning cup. One explanation
for the discrepant results could be the different methods used to assess results of the
cleaning.

Our study did not confirm the findings of Melin et al. (2002) that hind teats were cleaned
more effectively than fore teats with a cleaning cup because the robot arms seemed to
contaminate the fore teats while attaching the teat cups to hind teats. Neither did our work
confirm the opposite finding about teat location of Knappstein et al. (2004) who assessed
6 different methods of teat cleaning in AM. Our finding that cleaning the teat orifice was
less effective than cleaning the teat barrel or apex is critical because bacteria and sediment
on the teat orifice have direct access to the teat canal and they also end up in the raw milk
collected. According to Jørgensen (1990), the teat apex is the dirtiest part of the teat. It is
the part mostly in contact with milk during milking. According to our study, unsurprisingly, teat condition affected ETC. Rough teat ends and dry skin can also harbour bacteria (Neijenhuis, 2004).

6.2.3 Improving milking hygiene in automatic milking

The proportion of the technically successful cleanings should be much higher than found in our study. Teats should be reliably located before teat cleaning, and monitoring of the TSTC should produce lists of unsuccessful teat cleanings for the farmer. The substantial variation between herds represents ample reason to consider management modifications. This should be possible because one herd for which cup cleaning was used could have over 95% technically successful teat cleanings. If the proportion of successful teat cleanings is lower, management actions are required. Teat coordinates programmed into the computer should be re-evaluated, particularly in early lactation, when postpartum udder oedema decreases. MIs should be kept relatively regular, to maintain conformation of the udder. Cows with poor udder structure should be culled. Milking by the AMU should occur smoothly so that it does not cause distress through long waiting time for the robot or restlessness through unpleasant or painful milking due to incorrect machine settings. Laser lenses should be kept clean and bright, and udder hair should be regularly clipped. If these management actions are not enough, requesting technical service to address technical issues should be considered.

There is evident need for development of more effective, automated teat cleaning methods including drying the teats after cleaning. Focus should be kept on proper cleaning of the teat apex and orifice when developing methods for teat cleaning. Methods for determining teat cleanliness before cleaning should also be developed. Farmers should be informed about cows requiring manual cleaning and on resolving reasons for dirtiness. Adjustability of the brush- or cup-cleaning mechanism to suit herd or cow characteristics should be fully used, and the results carefully followed-up. After this study, teat cleaning methods have been developed further, but observations in practical situations on the farms have revealed no marked improvement in ETC.

Cleanliness of the teats before cleaning had a significant effect on the effectiveness of teat cleaning. As only approximately half of the extremely dirty teats became clean or almost clean during the automatic cleaning process, hygiene measures to improve the situation are important. Barn design and cow comfort have a great impact on cow and udder cleanliness (Cook, 2004). Roth et al., (2005) showed that less than 1 cubicle/cow and cows lying in the alleys, poor bedding management and quality, poor management and cleanliness of the teat cleaning device, poor claw health and no selection of cows for udder health were significantly associated with contamination of the teats. Consequently, experts on animal health and welfare should be involved in building and reconstruction of barns with AMS. Proper maintenance of the milking stable and automatic milking machine can do much to prevent teats from becoming soiled in the milking stall. Teat skin and orifices should be healthy to improve cleaning of the teats. The teat-cleaning device of the AMU should be clean, intact, and operated according to the recommendations of the manufacturer.
Finnish legislation (2006) currently demands regular inspection of the teat cleaning procedure and estimation of teat cleaning efficiency in AM. The functioning of the cleaning device can only be evaluated by observing teat cleaning of several cows. Wiping the teats with test cloths and visually assessing the results could be useful, simple and illustrative to demonstrate to the farmer teat cleanliness before and after cleaning at his farm. The method could also be used for research purposes.

6.3 Mastitis detection in automatic milking

6.3.1 Sensitivity of detecting mastitis using electrical conductivity

6.3.1.1 Subclinical mastitis

The sensitivity of EC in detecting quarters with subclinical mastitis was fairly low, agreeing with earlier research (Hamann and Zecconi, 1998; Biggadike et al., 2002; Bruckmaier et al., 2004). Only 40% of the quarters that had over 1 000 000 cells/ml milk were detected, even after lowering the detection thresholds in Group B. Quarters with a confirmed IMI generally failed detection, and only seldom had an alert. Van Asseldonk et al. (1998) carried out a study on expert opinions interpreting research results from commercial farms. SE and SP for detection of subclinical mastitis were estimated to be 58% and 82%, which would mean more TP and FP alerts than in our study.

Correlation between milk SCC and EC was low, as also shown earlier (Nielen et al., 1992; Bansal et al., 2005). Mean IQR for EC of subclinical quarters was lower than in previous studies, using on-line sampling (Norberg et al., 2004a). The IQR of EC for quarters with subclinical mastitis was well below the detection thresholds used in our study as well as in many other studies (Maatje et al., 1992; Landsbergen et al., 1994; Woolford et al., 1998). One reason for this may be the different milk fractions used: EC was measured continuously throughout milking in our study. Milk EC and SCC in the affected quarters are highest in the foremilk, which may have resulted in underestimation of the proportion of quarters classified as having subclinical mastitis using the continuous EC measurement in AM.

Using 100 000 cells/ml as the cut-off value for subclinical mastitis as recently suggested (Hamann, 2002), SE and NPV would have decreased accordingly in both groups. The numbers of FP would have slightly decreased in Group A. If the definition of subclinical mastitis had been IMI or IMI with SCC of ≥ 200 000 cells/ml, the number of cases would have decreased. However, SE would have improved, but not much, even though Nielen et al. (1992) suggested that IMI used as a gold standard resulted in the most accurate detection of mastitis with EC. They concluded that the current gold standard SCC reflects the defence status of the quarter, rather than destruction of cells and tight junctions indicated by EC. It may be true, as in experimental mastitis a rise in milk SCC from the challenged quarter was noted hours before the increase of EC (Milner et al., 1996;
Bruckmaier et al., 2004), and milk SCC remained high for several days longer than the increase in EC (Bruckmaier et al., 2004). Based on our results we conclude that questioning the use of milk SCC as a gold standard is not constructive, and EC seems not to be a good marker for subclinical mastitis where udder damage is not present to such extent as in clinical mastitis.

Our gold standard for subclinical mastitis was based on a single measurement, even though repeated measurements and conjoining of different tests have been recommended (Nielen et. al., 1995). The extended presence of high SCC in milk may better coincide with EC (Nielen et al., 1995), and better reflect the true health status of the udder (udder damage) (Nielen et al., 1992). We therefore tested different SCC levels to define subclinical mastitis; the more cells in milk, the greater the possibility of true subclinical mastitis, although SCC should not change dramatically over short periods (Biggadike et al., 2002). Higher SCC thresholds led to higher SE, although this was still not satisfactory. When measuring milk SCC on a single occasion, it is also common to use a wider time-window for EC alerts (Nielen et al., 1995; de Mol et al., 1997; Biggadike et al., 2002). However, when we looked back to 10 milkings before the test milking, SE was only slightly (max 17%) better than when comparing SCC and EC at a single milking.

6.3.1.2 Clinical mastitis

In our study, EC was not completely satisfactory in identifying clinically affected quarters because only fewer than 75% of the quarters had EC alerts, but is comparable to the average SE of milkers (70%-80%) to detect abnormal milk by visual observation (Hillerton 2000, Rasmussen, 2005). Supporting our results, in AM, 12 out of 29 (41%) clinical mastitis cases were detected with EC one day to one milking before the visual observation (Knappstein and Reichmuth, 2000). EC values in clinical mastitis correspond well with earlier findings (Hamann and Zecconi, 1998; Norberg et al., 2004). Mean IQRs for clinical mastitis were similar or a little lower than in the study by Norberg et al. (2004). In a meta-analysis of Maatje et al. (1992), with a higher IQR (on average 35%) for clinical mastitis, nearly 65% of the cases had an alert before clinical signs. However, in our study some quarters with clinical mastitis had IQRs similar to the healthy ones, and some quarters had no EC values. Norberg et al., (2004) showed that quarters with clinical mastitis can exhibit totally divergent EC patterns because clots in milk can interfere with the EC measurement, resulting in low mean EC and IQR.

On the basis of experimental mastitis trial results, milk EC starts to rise after SCC (Milner et al., 1996; Bruckmaier et al., 2004), but before clinical signs appear (Lake et al., 1992; Maatje et al., 1992; Milner et al., 1997). EC declines more rapidly than SCC (Bruckmaier et al., 2004). In our study, farmers detected clinical mastitis both before the AMS and after it. The exact time point at which clinical signs of mastitis first appeared was not defined and it is not possible to tell if detection of AMS was timely or not. It has been proposed that antibiotic treatment of mastitis could be started based on the so-called early warning system of EC (Milner et al., 1997; Hillerton and Semmens, 1999). This suggestion is, however, based on trial results, where the course of experimental IMI could be affected by treating the quarter with antibiotics immediately after the rise of EC. Our findings and results from other studies suggest that this practice is not advisable (Biggadike et al.,
2002). In a study on expert opinions on interpreting research results from commercial farms van Asseldonk et al. (1998) concluded that detection of clinical mastitis in AM (estimated to have a general SE of 73% and SP of 87%) under field conditions is inferior to that under experimental conditions. Furthermore, in the field it is in general very difficult to prove that preventive measures for mastitis taken based on EC alerts alone would really be worth the effort as it is not possible to know whether clinical mastitis would have been developed without the measures taken.

In the few cases of blood in the milk, EC did not react to the blood. Although Na\(^+\) and Cl\(^-\) ions come from blood (Hamann and Zecconi, 1998), the amount of blood in the milk may not have been high enough to induce higher EC in milk. Moreover, if passage of ions is related to the rate of recruitment of somatic cells into milk, as Bruckmaier et al. (2004) suggested, milk EC would remain low in many cases.

### 6.3.2 Sensitivity of detecting mastitis based on milk colour

Subclinical mastitis causes many changes in the composition of the milk, but they were not visually detectable in our study, at least by analysing milk colour. Colour index values did not differ between healthy and subclinical quarters, in agreement with Ordolff (2002), who reported that absolute colour values were not satisfactory for detecting milk with a high SCC, although milk colour was related to SCC and lactation stage. However, by measuring spectral reflection of quarter foremilk and calculating IQR, Wiedemann and Wendl (2003) detected up to 56% of the samples with > 500 000 cells/ml with an SP of 95%. The better results in their study may be linked to different thresholds used for detection and analysis of foremilk rather than continuous flow of milk.

The colour sensor system detected milk from quarters with clinical mastitis quite well, but did not outperform EC, based on this small sample of cases. However, 5 out of 17 cases of clinical mastitis were detected solely based on milk colour, though adding some value to the detection system. Ouweltjes and Hogeveen (2001) and Espada and Vijverberg (2002), using the same detection method as we used, showed that abnormal milk, colostrum and milk with blood had substantially lower colour values compared with normal milk. This was true in particular for the blue colour, which seemed to be most affected also in our study. However, not all cases of clinical mastitis were detected, and as pointed out by Ouweltjes and Hogeveen (2001); milk with clots in the foremilk only, without other visual abnormalities, had only small deviations in the colour (no statistical tests carried out).

In our study most instances of milk with blood were detected, but the alerts from the system were not always for bloody milk, but were based on the increase in the yellow colour in the milk. Whyte et al. (2004) detected all 6 cases with visually red milk (> 10 000 000 RBC/ml milk) with an optical on-line sensor. They demonstrated a SP of 99.6%, calculated from nearly 500 samples with fewer than 10 000 000 red blood cells/ml. Based on an experimental study, Rasmussen and Bjerring (2005) expected that systems would be able to detect most cases of visually detectable blood in milk under field conditions, although blood will appear in the milk by different patterns during milking, depending on the cause of haemorrhage. In our study, the results were promising with continuous measurement of blood in the milk.
6.3.3 Reliability of electrical conductivity and milk colour alerts

6.3.3.1 On the test day

SP for classifying healthy quarters correctly with the help of EC measurements was quite high for both AMS. However, as Sherlock et al. (2008) pointed out, SP is a confusing indicator for the detection performance, and suggested the use of “success rate”, which is equal to PPV, and “false alert rate”, which in this case was 0-32 false alerts/1 000 cow milkings, depending on the AMS (for cows having <200 000 cells/ml in all quarters). This means that 60 cows with 2.5 milkings per day, would receive nearly 5 false alerts/day in Group A and none in Group B, assuming the threshold of SCC would be 200 000 cells/ml. With the threshold of 400 000 cells/ml, there would be approximately 6 and 1.5 false alerts/day in Groups A and B, respectively. Group A had many FP alerts, resulting in a low PPV. Most FP alerts in Group A were from quarters from healthy udders (i.e., udders with no quarters with high SCC). Lowering the SCC limit for subclinical mastitis to 100 000 cells/ml would have increased SP and decreased the number of FP alerts in Group A, but this would happen at the expense of SE.

Although milk colour evaluation had a high SP, the other of the two alerts at test milking was FP. It came from a cow with another quarter having subclinical mastitis. In the study of Espada and Vijverberg, (2002), a cow with Str. dysgalagtiae infection in one quarter also had a changed reflection for the blue colour in the other quarters. According to Bansal et al. (2005), increasing evidence of interdependence between quarters has become available, i.e. mastitis in one quarter can affect the consistency of milk also in the other quarters.

6.3.3.2 During the follow-up period

Approximately 60% of all cows in Group A and 25% of the cows in Group B received alerts based on a high EC. By comparing the numbers of alerts per cow and per quarter it can be concluded that in Group A a considerable number of cows had alerts for more than one quarter, whereas in Group B this was rare. In the study of Biggadike et al. (2002), in 24% of the cow-weeks having alerts they were for more than one quarter. IQR was not used, but an EC value was compared with the mean of 14 previous measurements in their study. Different thresholds used in Groups A and B might be behind our finding.

The marked difference in frequency of EC alerts between fore and hind quarters in Group A could not be explained, although it seemed that there was a difference between milk SCC in the fore and hindquarters during the farm visit to Group A. Most of the alerts for the fore quarters in Group A and in all quarters in Group B were TP, but in Group A most of the alerts for the hind quarters were FP, usually assigned to healthy udders. With the test EC criteria (ie. tightening the threshold for Group A and loosening it for Group B), the difference in the number of alerts and in the proportion of TP alerts between the groups diminished. When a cow had alerts, usually 6-9 alerts/cow were recorded during the follow-up period in Group A, and 4-17 alerts in Group B, indicating that some pathological phenomena are affecting that quarter.
In Group B, colour alerts were more common than EC alerts at the quarter level. The proportion of cows with colour alerts revealed, however, that many quarters of the same cow often had colour alerts, which is understandable when colostrum milk is considered. Usually only 3 colour alerts/cow were noted during the follow-up period, which is occasional and indicates that some of the alerts where not truly related to abnormal milk or clinical mastitis. Indeed, most of the FP alerts were due to increased yellow colour in the milk. Milk colour can also vary according to the MI; the shorter the MI, the more yellow the milk (Espada and Vijverberg, 2002). Colour of milk also depends on milk composition and colour of milk fat, which in turn depends on the cows’ diet (Solah et al., 2007).

### 6.3.4 Improving mastitis detection in existing AMS

**6.3.4.1 What should be detected and when?**

Mein and Rasmussen (2008) claimed that most farmers would like the AMS to detect and separate abnormal milk, to inform the owner which cows should be treated for clinical mastitis or be culled, and identify the time point suitable for milk delivery after treatment. They also agreed with Kamphuis et al. (2008), in that abnormal milk and milk from a quarter with clinical mastitis should be subject to different classification models: one with a high SE and one with a high PPV. However, because abnormal milk mostly derives from clinical mastitis (IDF, 1999), it is hard to differentiate between the two. After an alert, the measures taken by the farmer (treatment, separation of milk) should depend on the clinical examination of the cow and milk, and bacterial analysis of milk. Milk with blood may require a different approach, however. As our studies show, yellow coloured milk is not always an indication of mastitis, and ideally the system should not react to it too easily. As to whether quarters with subclinical mastitis should be detected, we believe that most farmers would like to identify the quarters for economic (Halasa et al., 2009) as well as for herd health purposes. Some farmers might even like to set a certain level for their BMSCC (Poskiparta et al., 2009); not too low, but low enough to meet the requirements for premium milk quality. The terms of legislation and ISO-standard for AM (ISO/DIS 20966, 2007) should obviously also be met.

AMS have been given credit for providing early alerts for mastitis detection. This however is not very different from performing a CMT-test in CM because alerts are not given before any compositional changes in the milk appear, thus an “early alert” takes place at the stage of subclinical mastitis (i.e. based on an increase in SCC or EC). Of course, the CMT is carried out manually. The farmer has then a chance to examine the cow and take a sample for bacterial analysis, as well as to introduce possible preventive measures (e.g. frequent milking, preventing spread of the infection to other cows). If mastitis then turns clinical, treatment can be started. If the farmer does not pay attention to subclinical mastitis, early alerts are not necessary, because an early alert would lead to frustration and perhaps to ignoring the alerts because mastitis does not always progress to the clinical stage. Bennedsgaard et al. (2004) reported an increase in the use of antibiotic treatments for mastitis in 20 farms after the introduction of AM. Some of the farmers had increased
their treatment frequency because they trusted the alerts generated by the automatic mastitis detection system. This can be an issue of concern regarding the problems with FP alerts in our and in other studies.

6.3.4.2 Thresholds for electrical conductivity alerts

When the number of EC alerts was reduced by tightening the alert criteria in Group A, SE decreased to a certain degree, but the number of FPs was considerably lower. According to Maatje et al. (1992) and de Mol and Ouweltjes (2001), applying time-series models could lead to better performance in mastitis detection. Biggadike et al. (2002), achieved a SE of 40–54% to detect quarters with $> 200\,000$ or $400\,000$ cells/ml when they used 10-15% increase in EC as a cut-off for an alert, compared with average EC for the 14 preceding days. In our study, small-scale use of time-series was tested by taking the EC of the 3 last milkings (running average) into account. This reduced the number of occasional alerts due to sudden peaks in EC, and alerts originating from a decline of EC in the other quarters (Figure 15), which could occur due to solids attached to the electrodes or leakage of air from the teat cups (Barth, 2002). If this reduction in alerts leads to increased motivation on the part of the farmer to exploit the system, the change is justified.

A need to increase the number of EC alerts was noted in Group B. In the criteria for alerts, the IQR had to exceed the cut-off also in the previous milking in order to trigger an alert. With such criteria, no alerts may be created in quarters with variable EC (Figure 16), or quarters with many missing values, even if the average EC is much higher than in the other quarters. The SE of detecting mastitis was increased after removing this criterion. This criterion should not exist, and is no more used in current systems.

Comparison of quarters instead of the absolute EC values is preferable (Nielen et al., 1992; Jensen and Knudsen, 1991; Norberg et al., 2004) because it should eliminate the variation due to breed, parity, MI, DIM, milk temperature and composition (Hamann and Zeconci, 1998). In various studies (Maatje et al., 1992; Landsbergen et al., 1994; Woolford et al., 1998) IQR between the quarters with the highest and lowest EC in subclinical and clinical mastitis varied between 1.10 and 1.20. Here, an IQR of 1.15 in Group A resulted in considerably more alerts than an IQR of 1.20 in Group B. However, interdependence of the quarters has been shown for EC (Bansal et al., 2005) and SCC (Merle et al., 2007); EC or SCC in the milk of the healthy quarters of cows with one quarter with mastitis were higher than those in healthy quarters of a completely healthy cow. Hillerton and Walton (1991) also reported that subclinical S. aureus IMI in one quarter caused an increase in the EC in the other quarters, maybe because of toxin production and increased permeability. In spite of the interdependence, by measuring foremilk EC and SCC on a continuous basis (Jensen and Knudsen, 1991), most of the total variation in the EC was attributable to variation between quarters (44-65%), compared with variation from day to day or variation within a quarter. Norberg et al. (2004) also concluded that the best indicator for mastitis was IQR of the average level of EC, compared with the variation of EC during milking.
Figure 15. How to improve detection in existing models – Group A: In the figure electrical conductivity (μS/cm) values of 84 milkings of all quarters of one cow are presented. The following quarters were given alerts:

1) quarter marked with yellow at milking 9 (CMT 1)
2) quarter marked with light blue at milking 57 (CMT 1)
3) quarter marked with light blue at milking 82 and 84 (CMT 5)

When a running average was included in the criteria of an alert, only the two last occasions with CMT 5 had an alert.

Figure 16. How to improve detection in existing models – Group B: In the figure electrical conductivity (indexes) values and running averages of 67 milkings of all quarters of one cow are shown. The quarter marked with light blue (electrical conductivity) and blue (running average of electrical conductivity) had alerts at milkings 14, 27, 35 and 36 and a CMT of 4-5. When the criterion for increased IQR in preceding milking was excluded, more alerts were created (test criteria 3; 9 alerts, test criteria 4; 27 alerts). The suggestions generated by these examples should be tested in real situations in the field because the same data used here were used for testing and development.
Using an absolute EC value as a threshold may depend too much on normal biological variation (Norberg et al., 2004) and lead to too low SE or to many FP alerts, depending on the threshold. However, Sheldrake et al. (1983) found no improvement in mastitis detection using EC difference between quarters. In a meta-analysis of 17 studies, SE was highest when the absolute threshold of EC was used combined with EC difference between quarters (Nielen et al., 1992). In acute clinical mastitis, or if more than one quarter are infected, the use of a high threshold of absolute value is justified. In conclusion, both IQR and absolute value are useful thresholds in practice and could be and are used in combination in AM.

6.3.4.3 Using milk yield alerts

Nearly 85% of the cows and half of the quarters in Group A received alerts based on a declining milk yield during the follow-up period. Most cows had alerts in more than one quarter. Only 10-20% of the alerts were TP, which shows that this indicator was not adequate for mastitis detection, agreeing with Knappstein and Reichmuth (2000), who found that 149 cows during one year study got 770 FP alerts due to decreased milk yield. The logic behind the mathematics in the use of this indicator was not explained by the manufacturer, and on the alert list it was not obvious whether the alert was based on the milk yield or on EC. More effort had to be put into finding out the reason for the alert.

Milk yield could be a part of mastitis detection system when applying multivariate methods (Nielen et al., 1992). Knowledge based, multivariate methods used to interpret results from measurements would improve possibilities for distinguishing between healthy quarters and those with clinical mastitis (Hogeveen et al., 1991; de Mol and Ouwelijes, 2001; de Mol and Woldt, 2001) or with subclinical mastitis (Nielen et al., 1995; Sloth et al., 2003), but these have to date mostly been theoretical suggestions. In farms with low prevalence of mastitis, SP is usually high, but the number of FP may also be high (Landsbergen et al., 1994), and the different detection methods should be used in combination. If the milk yield drops, the farmer should examine the cow similarly as when the amount of feed consumed or the number of milkings/cow declines. Milk yield alerts should be clearly differentiated from mastitis alerts when used individually. At the moment, deviating milk yield (% of the expected yield) may still induce an alert, mixing mastitis detection list or changing the order of the alert cows on the list.

6.3.4.4 Treating missing values

Missing values of EC and milk colour resulted in missing alerts and diversions in our study. If the milk yield strongly declines, the meters cannot provide a value for these indicators. This may occur because of a very short MI, but also because of acute clinical mastitis. Missing values may also result from clots blocking the channels of the measuring equipment in clinical mastitis. Moreover, as milk solids may prevent the electrode from giving correct EC values, proper maintenance of the electrodes is important. De Mol et al. (2001) showed that in one farm, 25% of the cows milked conventionally received indeterminable EC values, and concluded that less than 5% would be a tolerable level.
Farmers should pay attention to missing measurements and establish the cause for them. In some systems missing values have been taken into account by colour coding on the alert list. This is preferable, and some fast system for the farmer to check the number of missing values for the day should be available. Missing values should not interfere with calculating running averages.

6.3.4.5 Interpreting the results to the farmer

In current AMS there are different ways of presenting the results to the farmer. Some examples are in the following:
1. Absolute values (EC, blood, and SCC), measured at cow or quarter level
2. Index values (EC, colour or combination of measurements), measured at cow or quarter level
3. Classes (SCC), measured on quarter level
4. IQR (EC) between the quarters

An alert is a farmer-friendly way of interpreting the results of a sometimes complex set of udder health traits by the AMS, using milk quality data and other cow-specific data. In current AMS there are also different ways of expressing an alert, including:
1. Verbal alerts explaining the reason for the alert
2. Symbols for alerts (brackets, asterisks)
3. Counters, where counter value tells the number of milkings with alerts
4. Colour coding depending on the number and frequency of alerts:
   - for new alert
   - for failed milking with alert on the previous milking
   - for an alert at last milking or decreasing number of milkings
   - an alert at many milkings or increasing number of milkings
5. Phone message when values above certain (high) thresholds have been detected

Using absolute values for EC, SCC and colour is the simplest and most easily accessible way of presenting the measurements of mastitis detection methods. However, according to the results of Poskiparta (2009), interviews of farmers showed that farmers having an AMS using classes prefer to have classes over some absolute values of SCC. This is understandable, given the tradition of using CMT. A prerequisite for using classes should be a scientifically justified allocation. Algorithms behind the index values are usually internal knowledge of the company, and not open for discussion or adjustments, which makes using them debatable. All means mentioned above for reporting an alert are useful, but attention should be paid to using them simply, understandably and logically. It should also be clear to the farmer, the grounds on which the alert is given.

Whether the results are presented as crude values, classes or IQRs and interpreted as alerts or, for example, probabilities or overall risks of mastitis, should be up to the farmer. Farmers should be made aware of the theory, basis and thresholds of the detection system. According to our own experience, this is not currently the case on farms. Researchers studying EC as a detection method for mastitis have pointed out that thresholds for alerts should be flexible between farms (Sheldrake et al., 1983, Hamann and Zeconci, 1998, Kamphuis et al., 2008) because of the biological variation of EC between healthy cows.
and between healthy herds (Hamann and Zecconi, 1998). Consequently, the farmer could choose between having more alerts with a greater precision and more FPs, or fewer alerts with lower SE, depending on the udder health status of the herd and other methods used to monitor udder health in that particular herd. For example, when herd prevalence of mastitis is high, test SE and PPV will be high (Hamann and Zecconi, 1998), and a test could be used for treatment decisions (Nielen et al., 1992), and even create a basis for automatic separation of milk (Nielen et al., 1995). With a low prevalence of mastitis, SE and PPV are much lower (Hamann and Zecconi, 1998), but a test would still have a high NPV, and could be used as a screening tool for mastitis (Nielen et al., 1992). According to a farmer interview (Poskiparta 2009), 9 out of 17 farmers using AMS model that have comparatively easy-to-understand and easy-to-adjust thresholds for alerts, have used the possibility of adjusting the thresholds, compared with a less adjustable AMS model (2/10 farmers). Furthermore, interpreting the results should be, at least for some extent, flexible. For example, a farmer could design his own alert list using parameters he prefers, thresholds found suitable and a coding system for the alerts he finds easiest to understand. Creating the algorithms on which to base the alerts is always a compromise, and the development of EC or any other milk related data could be followed up with time-series graphics for all quarters in the same figure (Figure 15 and 16). Different quarters would be easy to compare without alerts. Graphics can also help in the preliminary check of an “alert cow”.

6.3.5 New approaches to mastitis detection: Infra-red thermography

The LPS-model functioned as expected and clinical mastitis was similar to that described in many earlier studies (Hoeben et al., 2000; Lehtolainen et al., 2003). The thermal camera showed increased $T_U$ both in experimental and control quarters 4 h PC, in line with the rise in $T_R$. These results support the results of Bitman et al. (1984), who demonstrated that temperature of the peritoneal cavity and udder were closely correlated, and Metzner et al. (2008), who showed an increase of the udder skin temperature in experimental and control quarters 13 h after inoculation with *E. coli*. The thermal camera did not represent any diagnostic benefit because the increase in $T_U$ appeared later than systemic signs, local signs in the udder, or changes in milk appearance. The $T_U$ also normalised in parallel with $T_R$ during the first 24 h PC, but local signs and milk appearance reflected the inflammatory reaction continuing in the affected quarter until the end of the study period.

The finding that local signs were not detected as a rise in the surface temperature can be explained by the course of pathological changes during clinical mastitis. During mastitis, blood vessels of the udder dilate to bring blood cells to the site of infection (Jones and Plassmann, 2002). Hyperemia results in classical signs of infection: calor and rubor (i.e., heat and redness of the udder quarters). Permeability of the capillaries increases and plasma leaks into the interstitium, causing edema, seen as swelling (McGavin and Zachary, 2007), which, in turn, results in impaired blood circulation and decreased $T_U$. Unlike in mastitis in our study, local signs of inflammation were detected with IRT in laminitis of dairy cows and in myositis of the back muscles of horses (Nikkhah et al., 2005; Fonseca et al., 2006). Inflammatory processes without pronounced swelling may result in detectable increases in surface temperature, in contrast to the situation for
mastitis, where pronounced swelling was present. This was shown by Kemp et al. (2008) in field data; mastitic cows with local signs of the udder and no systemic signs had higher $T_R$ and increased $T_U$ differences between the quarters compared to cows without mastitis or cows with abnormal milk. Scott et al. (2000), on the other hand, took thermal images from the caudal aspect of the udder and detected a clear rise in temperature of the experimental quarter and a lower rise in the control quarter already 1 h after induction of mastitis with 10 µg of LPS, the same dose as used here. Rectal temperature did not increase earlier than 6 h PC in their study. The discrepancy between our results and theirs is not easily explained due to lack of details provided in their study.

Another possible explanation for the unsuccessful detection of the local signs could be the systemic effects of the LPS challenge. LPS acts as an exogenous pyrogen by producing cytokine in the mammary gland, which triggers an increase in the set point of body temperature of the hypothalamus. Through vasoconstriction of the peripheral blood vessels and shivering the animal tries to achieve the new set point temperature, leading to fever (McGavin and Zachary, 2007). The IRT showed an equivalent increase of udder temperature in the experimental and control quarters, reflecting a systemic effect of LPS. Local changes in the udder may have been hidden by the systemic effect, supporting Loughmiller et al. (2001), who suggested that IRT detection of the early stage of fever in pigs inoculated with *Actinobacillus pleuropneumoniae* might not have been possible because of vasoconstriction of the peripheral blood vessels.

The transient increase of $T_R$ accompanying experimentally induced mastitis was successfully detected with the help of a thermal camera, which recorded the temperature change in udder skin in all cows. IRT showed no promise however for early detection of clinical mastitis because $T_U$ was closely related only to $T_R$. This contrasts with the results of Hurnik et al. (1984), who were able to detect 4 out of 6 natural cases of mastitis from 1-3 d before the clinical diagnosis. In their study, detection was based on the expansion of the area enclosed by the temperature isotherm of 37°C in the gluteal region of the cow. Methods to detect signs of clinical mastitis were not described, and therefore no conclusions can be drawn from that study. The ability of IRT to detect naturally occurring mastitis automatically according to a common course of events, possibly starting with local signs of the udder, should be tested under field conditions (e.g., milking parlour or an AMS).

Measuring components from milk represents several challenges. Milk composition, including protein and fat concentration, varies from cow to cow and there is also intra-cow variation due to e.g. lactation stage of the cow. Milk composition also varies between different milking fractions and after different MIs in AM. Additionally, in AM, a cow with clinical mastitis may be too sick to visit the AMU voluntarily, resulting in a missing evaluation of the milk by the AMS. According to our results, scanning the udders with a thermal camera installed in a milking or feeding parlour or besides a walk-through alley could detect the presence of febrile diseases, including clinical mastitis. This could take place already before milking the mastitic cow. In addition, continuous monitoring of a herd with thermal imaging might bring additional value by detecting mastitis not only in lactating but also in dry cows. This would be an advantage as compared with monitoring systems associated with milking, but more data are needed to confirm this. Other means of monitoring the health of dairy cows by distant recording are computer-assisted cameras
and activity detectors, but they are not particularly related to AMS and are beyond the scope of this discussion.

Practical use of thermal camera would not be limited to mastitis. IRT could be used with low thresholds and consequently with high SE as a screening sensor for disease or oestrus detection, combined with animal calendar, feed consumption, cow activity and milk parameter data of the AMS. However, with high thresholds and consequently high SP, an increase in the temperature of a cow alone would always be an indication for clinical assessment of the cow. Schaefer et al. (2004) showed that febrile conditions were detected earlier from orbital scans than scans of the ear, nose, or back. In our study the udder proved to be a suitable site for the measurement. Udder hair is usually kept short for hygiene reasons. The $T_U$ reflected $T_R$ and the maximum temperature of the image were usable, even though not always situated on the actual inflamed quarter, as the highest temperatures were recorded from the groove between the udder and the hind leg.

According to Berry et al. (2003) circadian rhythm and exercise affected udder skin temperature. However, they concluded that IRT could be used for early detection of mastitis, when the environmental temperature is taken into account. Metzner et al. (2008) reported that the maximum diurnal variation of the mean udder skin temperature was <1°C and that the effect of ambient temperature was of minimal biological significance. Lying side of the cows might also affect udder skin temperature, depending on the surface of the stalls. If necessary these environmental effects can be accounted for by using proper modelling techniques. In addition, before the practical benefits and on-line usage of the system can be considered, more research must be conducted to find out the best location for the thermal camera in an AMS barn, to test the ability of the thermal camera to detect local signs of mastitis and mastitis of dry cows and pregnant heifers, to establish adequate detection thresholds and to create appropriate mathematical models for detection.

6.4 Maintenance of good udder health in automatic milking

Automatic handling of the cow during milking requires successful completion of a complex set of procedures. Milking frequency should be adapted for every cow, according to its state of lactation. Quarter-based milking reduces transfer of bacteria between the quarters of the same cow, but care should be taken to minimise contamination between cows by proper flushing or other handling techniques for milking equipment between the cows. Intramammary infections can spread from cow to cow via stalls, and preventing milk leakage is important, as well as grouping the cows according to udder health if possible. Effective teat cleaning and spraying of the teats after milking are additional means to prevent infections. AMU settings should be carefully monitored and adjusted to keep the teats in good condition. Mastitis detection should be reliable and timely, using the opportunities provided by the AMS, but also by screening cows with poor udder health, for example, for SCC every month. Awareness of bacterial distribution on the farm is further increased by taking bacterial samples of highSCC cows particularly after calving and before drying-off. With skilful management and careful observation of the cows, udder health can be maintained good or even improved in AM.
8 CONCLUSIONS

1. Udder health slightly deteriorated after a change from CM to AM. The effect was more pronounced than on CM farms that changed from tie stalls to free stalls.

2. AM herds had more new highSCC cows than CM herds during the first year after the change. There were no pronounced differences in individual cow SCC associated with AM and CM, and the increased BMSCC of AM herds in Finland is suggested to result mostly from diminished diversion of mastitic milk.

3. The technical success of teat cleaning and its efficiency were too low in AM.

4. Cow restlessness during milking, unsuitable udder conformation, long udder hair and technical problems in the operation of the AMS represent risks for failure of the teat cleaning process.

5. Good overall hygiene and good teat condition are important because < 70% of the extremely dirty teats became clean or almost clean in the automatic cleaning process.

6. Sensitivity of detecting subclinical mastitis was poor based on EC, but more than half of the quarters with clinical mastitis received alerts on the grounds of EC and milk colour. When both methods were used, nearly all quarters received alerts before or during the day the farmer observed clinical mastitis.

7. Using simple alert criteria manipulation, the high number of false positive alerts was decreased substantially. In herds using a different AMS brand, the low number of alerts almost doubled, slightly at the expense of the proportion of true positive alerts.

8. The increase in rectal temperature of cows with experimentally-induced clinical mastitis was successfully detected with the help of a thermal camera, but local inflammatory changes of the udder, which appeared already earlier, were not detected.
In the future at least the following aspects of AM should be investigated or developed:

1. The effect of infrequent milking on udder health.

2. The species distribution of mastitis-causing bacteria in AM and CM.

3. The effect of systematic failures in teat cleaning and teat cup attachment for some cows on udder health.

4. The effect of adjustments to the teat cleaning sequences on effectiveness of teat cleaning.

5. The means for automatic evaluation of teat cleanliness before and after teat cleaning.

6. More efficient detection methods for quarters with clinical and, in particular, subclinical mastitis.

7. The capacity of IRT to detect naturally occurring clinical mastitis under field conditions.

8. Improvements required to maintain better teat condition in some farms.

9. Effectiveness of flushing of liners to inhibit transfer of bacteria between cows.
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