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Cleaning and stimulation of teats in an Automated Milking Rotary

Spenrengöring och stimulering i en automatisk mjölkningskarusell

Louise Juhlin

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Abstract

In automatic milking systems (AMS) teat cleaning and stimulation of milk let-down are performed automatically in a standardized process before milking. The settings for teat cleaning must meet the requirements for hygienic quality of the milk delivered to the dairy plants. Moreover, the stimulation of milk let down must meet the requirements for high milking efficiency at farm level. The latest addition from DeLaval is the automatic milking rotary (DeLaval AMR™) which has the capacity to work with five robots on five cows at the same time. The AMR has different time settings for teat wash and time out time (TT), the time allowed for each wash station to finish the preparation of two teats. Depending on time settings that are used, milk hygiene and milking efficiency can be affected. The short term aim of the project was to optimize cleaning and stimulation of the teats before milking, and the long term aim was to improve milking hygiene to maximize milking efficiency and milk quality.

The project was carried out at the Swedish Livestock Research Centre at Lövsta, Swedish University of Agricultural Science in Uppsala. It was divided into two studies of which the first investigated how different teat wash times (WT) (3.5 s, 5.5 s and 7.5 s) affected teat cleanliness and milking parameters as incomplete milking, attachment failure, kick-off, milk yield, milking time and milk flow in the DeLaval AMR™. The second study investigated how different TT (25 s and 30 s) using a WT of 5.5 and 7.5 s affected teat cleanliness and milking parameters using the same outcomes as in the first study. In both studies, all cows included were subjected to all treatments in a crossover design.

In the first study, it was found that the probability of high hygiene scores, i.e. dirty teats, was significantly lower after WT 5.5 s and 7.5 s compared to after WT 3.5 s. The probability of high hygiene scores was also significantly lower after WT 7.5 s than after WT 5.5 s. Results also showed that WT had significant effect on milking parameters. The risk of incomplete milking and attachment failure was higher after WT 3.5 s compared to WT 5.5 s and 7.5 s. A lower milk flow was found after 3.5 s WT compared to 5.5 s and 7.5 s WT. Significant differences in incomplete milking, attachment failure and milk flow were not found between WT 5.5 s and 7.5 s. In the second study, significant differences in hygiene assessments, incomplete milkings or attachment failures were not found between the two TT tested. A TT of 25 s, resulted in more kick-offs compared to TT 30 s when the WT was 5.5 s. However, the same results were not found when using a WT of 7.5 s.

It was concluded that a WT of 7.5 s resulted in best teat hygiene score. It was also concluded that the teat WT before milking cannot be shortened from default 5.5 s to 3.5 s (default TT 30 s) without risk for deterioration of the teat hygiene and milking parameters studied. Moreover, when using WT 5.5 s the TT can probably be shortened from 30 s to 25 s without deterioration of the teat hygiene and milking parameters studied. However, further assessment of the effect on teat hygiene is needed since teat hygiene was only evaluated on the right side of the udder.

Sammanfattning

Rengöring av spenar och stimulering av mjölknedsläpp sker med automatik i automatiska mjölkningssystem (AMS). Således krävs det att inställningar för spentvätt uppfyller kraven på hygienisk kvalitet på mjölken som levereras till mejeriet. Vidare måste också stimulering av mjölknedsläpp uppfylla kraven för hög mjölkningseffektivitet på gårdsnivå. Det senaste mjölkningssystemet från DeLaval är den automatiska mjölkningskarusellen (DeLaval AMR™). AMRen har olika inställningar för bland annat spentvättid och ”time-out”-tid (tiden det tar för varje tvättstation att rengöra två spenar). Beroende på vilka tidsinställningar som används i AMRen kan mjölkningshygien och mjölkningseffektivitet påverkas. Syftet med den här studien var att, på kort sikt, optimera rengöring och stimulering av spenarna innan mjölkning, och det långsiktiga målet var att förbättra mjölkningshygienen för att maximera mjölkningseffektivitet och mjölkkvalitet i system med automatisk mjölkningskarusell.

Projektet genomfördes vid Nationellt forskningscentrum för lantbrukets djur, Lövsta, Sveriges Lantbruksuniversitet, i Uppsala. Den första delstudien undersökte hur olika spentvättider (3,5 s, 5,5 s och 7,5 s) påverkade spenhygienen och olika mjölkningsparametrar så som ofullständig mjölkning, misslyckade påsättningsförsök av spenkopp, avspark av spenkopp, mjölkmängd, mjölkningstid och mjölkflöde i en DeLaval AMR™. Den andra delstudien utvärderade hur olika ”time-out”-tider påverkade spenhygienen och samma mjölkningsparametrar som i den första studien. I båda studier utsattes alla inblandade kor för samtliga behandlingar i en ”cross-over design”.

Resultat från den första delstudien visade att sannolikheten för höga hygienpoäng, dvs smutsiga spenar, var signifikant lägre efter 5,5 s och 7,5 s tvättid i jämförelse med 3,5 s tvättid. Sannolikheten för höga hygienpoäng var också signifikant lägre efter 7,5 s tvättid i jämförelse med 5,5 s tvättid. Risk för ofullständig mjölkning och misslyckade påsättningsförsök av spenkopp var högre efter 3,5 s tvättid i jämförelse med 5,5 s och 7,5 s tvättid. En tvättid på 3,5 s visade sig ge lägre mjölkflöde i jämförelse med 5,5 s och 7,5 s. Det var ingen signifikant skillnad på ofullständig mjölkning, misslyckade påsättningsförsök av spenkopp och mjölkflöde vid jämförelse av 5,5 och 7,5 s tvättider. I den andra delstudien användes två olika ”time-out”-tider, 25 och 30 s, med tvättid på 5,5 och 7,5 s. Inga signifikanta skillnader i varken hygienbedömning, ofullständig mjölkning eller misslyckade påsättningsförsök av spenkopp mellan de olika time-out-tiderna fanns. Däremot resulterade en time-out-tid på 25 s i fler avspark av spenkopp i jämförelse med 30 s time-out-tid, när tvättiden var 5,5 s. Denna skillnad fanns inte med en tvättid på 7,5 s. Det konstaterades att en tvättid på 7,5 s resulterade i högst hygienpoäng.

Spentvättiden innan mjölkning kan inte förkortas från standardtiden 5,5 s till 3,5 s (30 s ”time-out-tid”) utan att riskera en försämring av spenhygienen och de mjölkningsparametrar som studerades. Time-out-tiden kan eventuellt förkortas från 30 s till 25 s med en tvättid på 5,5 s utan att försämra spenhygienen och mjölkningsparametrar. Detta kräver dock vidare utredning eftersom endast spenarna på den högra sidan av juvret hygienbedömdes i de två delstudierna.

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Abbreviations

AM - Automatic milking

AMS - Automatic milking systems

AMR - Automatic milking rotary

CM - Conventional milking

CMS - Conventional milking systems

DIM - Days in milk

K1 - Cow group nr 1

K2 - Cow group nr 2

K3 - Cow group nr 3

RF - Right front teat

RR - Right rear teat

SCC - Somatic cell count

SH - Swedish Holstein

SR - Swedish Red

TOF - Time of flight

TPM - Teat preparation module

TT - Time-out time

WT - Wash time

1. Introduction

The Swedish dairy industry has experienced great changes during the past decades resulting in a fast decrease in the number of farms, and a substantial increase in the number of cows per farm (Gustavsson, 2010; Växa Sverige, 2015). Moreover, automatic milking systems (AMS) have become common. In such systems, teat cleaning and stimulation of milk let-down before milking are performed automatically following standard settings. However, the settings for teat cleaning needs to meet the requirements for hygienic quality of the milk delivered to the dairies and stimulation of milk let down has to meet the requirements for high milking efficiency at farm level.

The automatic cleaning of the teats is a standardized process, meaning that the cleaning cannot be adjusted to the dirtiness of the individual teat (Dohmen, 2010). Thus, it is the effectiveness of the teat cleaning unit that determines the result of the cleaning (Hovinen et al., 2005). It has been observed that total bacterial count and numbers of anaerobic spores are higher in bulk milk from AMS farms compared to in bulk milk from farms with conventional milking (Klungel et al., 2000; Van der Vorst and Hogeveen, 2000; Rasmussen et al., 2002) indicating that contamination of the milk from teat surface is more common in AMS farms. Several studies have also shown that cows in AMS have higher milk somatic cell count (SCC) compared to cows in conventional systems, which indicate deterioration of udder health and changes of milk composition that can have adverse effects on milk quality (Van der Vorst and Hogeveen, 2000; Kelton et al., 2001; Rasmussen et al., 2002; Van der Vorst and de Koning, 2002). However, other studies have shown a decrease in milk SCC (Nogalski et al., 2011) or no differences in SCC (Klungel et al., 2000) when comparing conventional milking (CM) to automatic milking (AM).

In all dairy farms a high milking efficiency (cows milked per hour or litre milk harvested per hour) is warranted, which is accomplished by optimising milking routines. One important part of those routines is stimulation of the teats to induce milk ejection, in which the pituitary hormone oxytocin is involved. Teat stimulation has been found to have a significant influence on milk flow and milking time (for review see Svennersten-Sjaunja, 2004) and thereby on milk yield. In AMS where the teat stimulation is performed by a robot, it is very important that there is no disturbance in milk ejection, in order to contain a high milk flow which in turn will have a positive effect on milk yield and milking time.

Recently, the Automatic Milking Rotary (DeLaval AMR™) has been introduced into the market. In this system cleaning and stimulation of teats, including removal of foremilk, are performed during a specified time period using a special wash cup in two wash stations. The system allows a few different settings for the wash time (WT) and the time allowed for each wash station to finish the preparation of two teats, known as the time-out time (TT). By keeping the WT and/or TT as short as possible, the milking efficiency in the AMR can possibly be improved since the number of cows per time unit in the AMR will be reduced. A reduction of the WT and/or the TT could, however, have negative effects on milk and teat hygiene if the teat cleaning is insufficient. Insufficient teat cleaning may also increase the risk for attachment failure (Kolbach et al., 2013). Shorter WT and/or TT may also have negative effects on milking efficiency due to reduced stimulation of milk let down. In a study by Dzidic et al. (2004), it was found that the use of the teat cleaning device (5 seconds (s) of washing) in the Voluntary Milking System (DeLaval VMS™) was sufficient to induce milk ejection. However, to my knowledge, there are no studies on the impact of different WT and TT in an AMR on milking efficiency or teat hygiene.

1.1 Aims and hypotheses

The short term aim of the project was to optimize cleaning and stimulation of the teats before milking, and the long term aim was to improve milking hygiene to maximize milking efficiency and milk quality.

It was hypothesized that the cleaning and stimulation time, i.e. WT, before milking cannot be shortened, relative default settings, without risk for deterioration of the teat hygiene and milk flow, and that the TT of the teat preparation module (TPM) at cleaning can be shortened somewhat without causing significant deterioration in teat hygiene and milk flow.

2. Literature review

2.1 Occurrence and function of automatic milking

AM was commercially launched in the Netherlands in 1992, and in 1998 the first AMS was installed in Sweden (Benfalk and Gustavsson, 2004). In 2010, nearly 20% of the Swedish dairy cows were milked in AMS. The number of farms with AM is increasing, not only in Sweden but also worldwide (Växa Sverige, 2015). Among the recognized benefits of AM lies reduced labour (Hogeveen et al., 2004; Gustavsson, 2010) and increased milk yield due to higher milking frequency (Løvendahl and Chagunda, 2011; Soberon et al., 2011; Hart et al., 2013). On the contrary, disadvantages like high investment costs (Gustavsson, 2010) and inferior milking hygiene (Klungel et al., 2000; Hovinen, 2009) are addressed in several reports considering the comparison between AMS and conventional milking systems (CMS).

Since there are several brands of AMS on the market, there are also some differences in the construction of the milking devices and gate systems. In general, AMS is a complete management system that involves one or several milking units, comprising milking machine, teat preparation device and gate systems in order to control cow traffic. The system is monitored through a computer, in which all cow data is available for the farmer (Gustavsson, 2010). When the cow enters the milking unit, an ID sensor reads the identification tag on the cow and "decides" whether the cow should be milked or not, depending on the cow status. If the cow has been milked recently, she will be redirected from the milking unit. The gates can also be used in order to transfer cows for other purposes (Gustavsson, 2010), for example insemination or treatment. As cows are rarely motivated to be milked, concentrate is often provided in the milking station in order to motivate the cow to visit the milking unit (Prescott et al., 1998; Koning and Rodenburg, 2004).

Teat cleaning in AMS can either be performed with a separate washing teat cup, within teat cups used for milking or by brushes. Removal of foremilk is done simultaneously with teat cleaning in which cleaning water and foremilk are separated from the bulk milk (Knappstein et al., 2004). There are also some differences in teat cup attachment depending on AMS brand. However, both attachment/detachment of teat cups and application of post-milking teat disinfectant are automatically performed in all of the AMS brands. In all brands, AM is quarter based, which reduces the risk of spreading intra-mammary infections between teats within cow (Hovinen, 2009).

Regarding cow traffic, there is quite a large variation in systems used on farms. This is also due to AMS brand, design of the stall and preference of the farmer. The cow traffic can either be free, selective or forced (Melin et al., 2007). In all AMS, the cows are housed in free-stall houses.

One-booth robot(s) (e.g. DeLaval VMS™ and Lely Astronaut A4), multi-booth robots (e.g. Mlone and SAC RDS) and DeLaval AMR™ are three different systems that provides complete automation during milking. One-booth robots are suitable for a maximum of 65 cows per milking unit (Sällvik, 2015) and AMR is suitable for larger farms (>300 cows) (DeLaval, 2015).

2.1.1 Function of automatic milking rotary

The DeLaval AMR™ is able to milk 90 cows per hour when installed with five robots. In total, it is possible for four robots to work on four cows at the same time. Additionally, a fifth robot can perform disinfection of the four teats after completed milking. The system is suitable for different types of milk production system such as loose house system or pasture based dairying. Depending on the management approach on the farm the capacity of the rotary can be utilized in different ways. This applies to number of milkings a day and number of robots installed (DeLaval, 2015).

At the entrance gate the electronic ID of the cow is scanned. The cow enters the rotary and will then have the same location on the platform during the entire milking. When she has been moved to the second step in the rotary the two rear teats are prepared by being washed a few seconds, dried and pre-milked by one robot. At the third step, a second robot is doing the same thing with the front teats. During the following two steps teat cup attachment is performed on the rear and front teats by two robots, starting with the rear teats. Then, the actual milking starts. All robots are equipped with a time of flight (TOF) camera that views the object in 3D and measures the time it takes for the light to hit the object. In this way the robots are able to locate the teats in real time. The udder is milked on udder quarter level, which means that milk flow, total milk yield, blood occurrence in milk and milk conductivity is measured for every udder quarter separately. When the milk flow declines below a pre-set milk flow, the teat cups are automatically detached. Before the cow leaves the rotary the four teats are disinfected by one robot. For hygienic reasons the system is equipped with an automatic deck flush module consisting of a scraper blade and water jets that can keep the manure out of the platform. After finished milking the cow will pass the selection gate and enter the lying and feeding area. If the cow is incompletely milked, she can be redirected to the rotary for a second milking attempt (DeLaval, 2015).

2.2 Milking hygiene and teat cleaning in automatic milking

The hygiene of milk for human consumption must be controlled closely to protect consumers from illness caused by bacteria and other microorganisms. Despite the fact that all milk sold in store is pasteurized in Sweden (Livsmedelsverket, 2015), good hygiene control before and during milking is necessary to produce milk with good quality (Pankey, 1989; Rasmussen et al., 1991) and processability.

Having clean and dry teats before milking not only reduces the risk of contaminating the milk with environmental bacteria, it will also decrease the risk of intra-mammary infections of the cow (Pankey, 1989). In CMS the milker has the opportunity to ensure good udder hygiene as the teat/udder inspection is done prior to attachment of the milking cluster. Dirty teats will get more attention to make sure that good teat hygiene is maintained. In AMS the teat preparation is programmed and all teats are cleaned for the same length of time with the same intensity, regardless of degree of teat dirtiness (Ten Hag and Leslie, 2002) and there is no visual assessment before the teat cups are attached (Hvaale et al., 2002). Thus, whether the teat is in the cleaning device during cleaning and is truly cleaned is not detected by sensors in any current AMS (Hovinen et al., 2005), which increases the risk of bacterial contamination of the milk.

2.2.1 Teat cleaning in different cleaning devices

As mentioned above, different AM brands have different types of cleaning devices. Therefore, the cleaning efficiency might be different depending on which type of washing device is used (Knappstein et al., 2004). Compared to manual cleaning it has been reported that cleaning in AMS was less effective (Knappstein et al., 2004; Tangorra et al., 2004), but in another study it was more effective (Melin et al., 2002). However, the study by Melin et al. (2002) was a controlled trial and the teats were dipped in slurry contaminated by spores. Knappstein et al. (2004) reported that the bacterial contamination was higher after cleaning compared to before cleaning at some farms, which was suggested to be due to insufficient disinfection of the teat cleaning device.

In a study conducted by Hovinen et al. (2005), the effectiveness of teat cleaning was evaluated when comparing two AMS brands using either a separate teat wash cup or rotating brushes during teat preparation. It was shown that using the teat wash cup resulted in a significantly larger proportion of clean or almost clean teats compared to cleaning with brushes (79.8 versus 72.9%). Almost clean and slightly dirty teats were cleaned well, but dirty and extremely dirty teats were not cleaned sufficiently, approximately 45% were left dirty, in both systems. At the visual assessment, almost all teats had the same hygiene score or were cleaner after the cleaning step compared to before cleaning independent on treatment (Hovinen et al., 2005). As reported by Ten Hag and Leslie (2002) there were no difference in numbers of teat skin bacteria when comparing manual cleaning and cleaning by brushes. Compared to the teat apex and barrel, the area around the teat orifice was less effectively cleaned, which is consistent with results from Knappstein et al. (2004). Both studies concluded that a dirty orifice is critical since bacteria in that area are in direct contact with the teat canal.

Knappstein et al. (2004) also compared the teat cleaning effectiveness of different brands of AMS by artificial contamination of teats with a tracer substance (poppy seeds mixed in manure) that could be transferred from the teat surface into the milk. For each brand, five contaminated cows were milked without previous teat cleaning and five other contaminated cows were milked after teat cleaning. The results showed that the teat cleaning efficiency varied markedly (from 50-70% to more than 85% reduction) between brands. The authors concluded that the teat cleaning on the farms might be even less effective since the teat cleaning in the experimental setting occurred less than one hour after the application of tracer substance and the adhesion of bacterial spores and manure is probably stronger than adhesion of poppy seeds.

2.2.2 Cow factors affecting teat cleaning

Factors related to the cow might influence the efficiency of the automatic teat cleaning process. Unsuccessful teat cleanings in AMS were more common in cows with excessively long udder hair than in cows with less udder hair due to attachment of bedding material to the udder hair (Hovinen et al., 2005). In the same study it was also found that black teat pigmentation in one of the groups were associated with unsuccessful teat cleaning. In this group the cleaning device used a laser in order to find the teat during teat wash cup attachment.

Hvaale et al. (2002) found a connection between poor udder shape and unsuccessful teat wash. There was a tendency that the teat cleaning with washing cup more easily managed the disparate udder shapes compared to teat cleaning with brushes.

2.2.3 Effects of management practices on teat hygiene

Bacteria from the environment, milking equipment (Pankey et al., 1987) and cows with intra-mammary infection (Schreiner and Ruegg, 2003) can cause udder infections and mastitis. *Staphylococcus aureus*, is the most common contagious udder pathogen in Sweden and is usually spread from infected cows to healthy cows via the milker or milking machine (Phillips, 2009). In contrast, environmental udder pathogens, like *Escherichia coli,* are mainly spread from the environment to the udder between milking occasions. Thus, it is important to, as far as possible, keep the udders clean. Good udder hygiene can mainly be achieved through good farm management practices, such as keeping cubicles and floors clean, using sufficient amounts of clean bedding material, and keeping udder hair short (Hovinen et al., 2005). According to Magnusson (2007) hygiene scores of teats and udders were improved when faecal contamination of the cubicles was reduced.

In the trial by Veissier and Capdeville (2004) it was found that cow cleanliness was affected by cubicle characteristics. For example, cubicles with a high positioned neck rail as well as short or narrow cubicles (in relation to recommended dimension) had adverse effects on the cows' ability to use the cubicle. A narrow cubicle forced the cow to lie down with part of their body in an adjacent cubicle. The same thing was found for cows with less space for their head (Veissier et al., 2004). In consequence of a sidelong position in the cubicle, there is a higher risk for manure to end up on the lying area, which increases the risk of contamination of the udder of the cow.

2.3 Stimulation of milk let down and milking efficiency

Activation of the milk ejection reflex is required in order to milk a cow sufficiently. The hormone oxytocin is released from the posterior pituitary as a reaction to tactile teat stimulation performed by hand, calf or milking machine. Oxytocin causes contraction of the myoepithelial cells that surrounds the small ducts and alveoli of the mammary glands after which milk is released (Ely and Petersen, 1941). In addition to tactile stimulation of the teats and udder, olfactory, auditory and visual stimuli can also induce oxytocin release and milk let-down (Sjaastad et al., 2010).

In the absence of oxytocin, the milk ejection is inhibited, which will result in production losses. It is important that milk ejection occurs before the udder cistern is emptied, since milk removal on an "empty teat" can lead to disturbance of further milk removal. In order to maintain a high level of milk synthesis during the ongoing lactation, it is required that the alveolar milk is removed during milking. This can only be achieved with complete milk ejection (Bruckmaier and Wellnitz, 2008). However, there is always some residual milk left in the cisterns/alveoli after milking.

In a dairy cow approximately 80% of the milk is stored in the alveolar compartments and only 20% in the cisterns. The cisternal milk fraction is mainly disposable for pre-milking and for the suckling calf before milk ejection (Bruckmaier and Blum, 1992). When milking without pre-stimulation the milk ejection reflex is delayed and milk flow can be adversely affected. The milk flow can either be temporarily decreased or interrupted after the cisternal fraction is removed if the milk ejection is not initiated (at the onset of milking). Late milk ejection can cause so called bimodal milk flow curves which can have negative effects on milking efficiency by causing a prolongation of machine-on time (Bruckmaier and Blum, 1996).

2.3.1 Teat preparation and teat cup attachment – effects on oxytocin release

It has been observed that pre-milking teat cleaning devices in AMS effectively induce oxytocin release and activate the milk ejection which is needed for optimal udder emptying during milking (Bruckmaier et al., 2004; Dzidic et al., 2004). As described by Dzidic et al. (2004) the oxytocin concentration was found to be lower when no pre-milking treatment was performed before the start of the teat cup attachment compared to teat preparations with either cold water, warm water and preparation with warm water in two cleaning cycles (122 s versus 58-60 s) before milking. However, the oxytocin concentration rose within 30 s after teat cup attachment in the no pre-milking treatment group. It was found that teat preparation with both cold and warm water was suitable for inducing milk ejection. In a study by Mačuhová et al. (2004) delayed teat cup attachment and long lasting teat cup attachment (removal and reattachment of teat cups with or without subsequent milking) had no negative effect on oxytocin release and milk removal during subsequent milking in a multi box AMS.

2.3.2 Teat preparation - effects on milk flow

The recommended time for pre-stimulation extends between 20-90 s in CMS, depending on degree of udder fill. A high degree of udder fill needs less time for pre-stimulation, while a low degree of udder fill needs longer time for pre-stimulation of the milk let down. In that time span an immediate and continuous milk flow was guaranteed at the onset of milking in order to receive total milk removal (Weiss and Bruckmaier, 2005). Given the information above, milking without pre-stimulation can have an adverse effect on milk flow, since the milk ejection reflex can be delayed. Therefore, it is of importance to be sure that total milk ejection occurs even during automatic teat stimulation, which will be induced by a robot.

In a study conducted by Dzidic et al. (2004) four different treatments of teat preparation was compared in a VMS; no pre-milking preparation, cold water preparation, warm water preparation and preparation with warm water in two cleaning cycles (122 s versus 58-60 s). It was shown that the average milk flow was significantly lower up to 8 hours from the previous milking for teat preparation without pre-milking compared to the other treatments. Peak flow rate did not differ between the treatments. As described by Davis et al. (2008), a greater mean quarter milk flow rate was observed after the wash treatment compared to no wash treatment.

Kolbach et al. (2013) found no significant differences in average milk flow rate when comparing cows subjected to wash treatment and cows with no wash treatment before milking. However, it was found that cows subjected to the wash treatment with a milking interval ≤8 h had significantly higher peak flow rates (300 g/min increase) compared to cows with no wash treatment in the same milking interval group. Weiss and Bruckmaier (2005) found the highest peak flow rate when the duration of pre-stimulation was longest, i.e. 90 s. Also, the average flow rate during main milking was increased when the duration of pre-stimulation was prolonged for udders that were not full.

2.3.3 Teat preparation - effects on milk yield

Since teat preparation is of importance for efficient milk ejection it is likely that teat preparation affects milk yield. However, different results have been found in studies. Cows who were subjected to either teat wash (5.5 s of cleaning/teat) or no teat wash in a VMS, showed no significant effect in milk yield per milking session between the treatments (Davis et al., 2008). As described by Jago et al. (2006), no differences in milk yield per milking or yield per day were found when comparing teat cleaned with brushes and no cleaning with brushes. Sandrucci et al. (2007) found that proper teat preparation, including fore stripping and pre-dipping, had a positive effect on milk yield, milk flow rate, less bimodality of milk flow curves and shorter total milking time compared to poor teat preparation. However, this study was made in CMS.

2.3.4 Teat preparation - effects on milking time

In a study conducted by Weiss and Bruckmaier (2005) the main milking time, without pre-stimulation and machine stripping, was shown to be shortest when the duration of pre-stimulation was longest, i.e. 90 s, and longest when there was no pre-stimulation. Yet, the total milking time, including pre-stimulation and stripping was shortest without pre-stimulation. However, this study was done in a tandem milking parlour using vibration stimulation. As described by Davis et al. (2008), the average crate time (total time spent by cows in the milking unit) per milking session per cow was 1.1 minute longer for cows with wash treatment compared to cows with no wash treatment. Dzidic et al. (2004) found that the main milking time was longer for cows with no pre-milking teat preparation compared to cows with cold water preparation, warm water preparation and preparation with warm water in two cleaning cycles (122 s versus 58-60 s). Jago et al. (2006) found that the milking time was 0.54 minutes longer, on average, if teats were cleaned with brushes compared to if teats were not cleaned before milking. However, the cups-on-time (time from attachment of the first teat cup to removal of last teat cup) per milking was reduced when cows were cleaned with brushes before milking compared to when not cleaned with brushes. The milk harvesting was also 2.9 s/kg faster when milkings were preceded by teat brushing compared to milkings without pre-treatment.

In order to provide a high milking efficiency, it is important to obtain a high milk flow and a rapid milking time, mentioned above. A shorter milking time per cow may increase the milking efficiency, which has economical advantageous for the farmer. It may also decrease the waiting time in the collection area.

2.4 The importance of avoiding disturbance during milking

As it is important to receive a high milk flow and short milking time in order to improve milking efficiency, it is also important to avoid disturbance like teat cup attachment failures, kick-offs and incomplete milking during milking. Otherwise, the milking procedure might be prolonged which has economical disadvantages for the farmer. It may also increase the cow waiting time before milking.

2.4.1 Teat cup attachment failure

In a study conducted by Kolbach et al. (2013) a trial was made to investigate whether the attachment of the teat cup was more successful if the teat was washed in a teat preparation module compared to not being washed in a prototype AMR. Results showed that the teat cup attachment had up to 1.5 times higher odds of being successful if the teat was washed in a teat preparation module compared to if the teat was not washed. The teat cup attachment was also faster if the teat was washed before teat cup attachment. On the contrary, Jago et al. (2006) found no significant differences in teat cup attachment success after teat cleaning with brushes compared to after no teat cleaning in a one-booth robot. The reason for the different findings in the two studies might be differences in the number of attachment attempts in AMR versus one-booth robot. In an AMR (Kolbach et al., 2013), the robot has only one attachment attempt compared to several attempts in the one-booth robot (Jago et al., 2006). The major problem in teat cup attachment in AM is the distance between rear and front teats. Moreover, Kolbach et al. (2012) reported that failures in teat cup attachment were more likely on left rear teats compared to right front teats in the AMR, but reasons for this was not known.

According to a study by Bach and Busto (2005) the occurrence of teat cup attachment failures in a one booth robot was 7.6% of the total milkings. The failures depended primarily on the ability of the milking teat cups to locate the teats even though the washing teat cup was successfully attached. Thus, all quarters were stimulated in the study, but were in some cases not milked. The same study also found a reduction in milk production after teat cup attachment failure, and that the reduction was larger with increasing days in milk (Bach and Busto, 2005).

2.4.2 Incomplete milking

When milking of a cow is registered as incomplete in the AMR, the cow will be redirected to the platform in order to complete the milking. The definition of incomplete milking is when the milk yield of a cow is less than 50% of expected yield (Delaval, 2015). Hamann et al. (2004) found that incomplete milkings in a one-booth robot were due to technical as well as cow factors. In 70% of the cases only one quarter was incompletely milked (Hamann et al., 2004). Since the robot arm that attach the milking cluster does not follow the cow during the entire milking in the AMR, the unattached or pre-term removed milking cups cannot be (re) attached once the cow has passed the teat cup attachment station. This might increase the risk of incompletely milked cows in AMR (Kolbach et al., 2012). However, manual reattachment can be performed in the AMR. The definition of incomplete milking in the study by Kolbach et al. (2012) was when not all teats were attached successfully for milking. Kolbach et al. (2013)

suggested that incompletely milked cows could have more complex udder confirmations, since only one or more quarters per cow were found to be incompletely milked in that study. It was more likely for multiparous cows to have successful milkings compared to primiparous cows, which the authors explained by differences in animal behaviour but also in the shape of the udder (in younger cows the udder was more compact and higher than in older cows) (Kolbach et al., 2012).

3. Material and method

The project was carried out at the Swedish Livestock Research Centre at Lövsta, Swedish University of Agricultural Science in Uppsala. A pilot study was performed in October 2014 to investigate if the intended methods were implementable. The likely proportion of cows per group that was possible to study at each milking was also estimated. The project was approved by the Uppsala Ethical Committee, and were performed between November 2014 and January 2015. The project was divided into two studies of which the first investigated how different teat WT (3.5 s, 5.5 s and 7.5 s) affected teat cleanliness and milking parameters as incomplete milking, attachment failure, kick-off, milk yield, milking time and milk flow. The second study investigated how different TT (25 s and 30 s) affected teat cleanliness and milking parameters using the same outcomes as in the first study. TT is the time for each TPM to finish the preparation of two teats. In both studies, all cows included were subjected to all treatments in a crossover design. In addition, before the start of each study teat hygiene assessment of the cows was made once before they entered the AMR to see if the hygiene scores differed between the cow groups.

3.1 Housing and management

The cows were housed in a free stall barn divided into three sections, K1, K2 and K3 containing 64, 58 and 58 cubicles, respectively. All cubicles were covered with rubber mats. The lying area was automatically sprinkled with bedding material (shavings) twice each day. Three times a week the end of every cubicle was manually sprinkled with Agrosan DryOut (Vilomix Sverige AB), which has a disinfecting effect against bacteria and fly larvae by absorbing moisture and binding ammonia. In section K1 all concrete floors were covered with rubber mats. In sections K2 and K3, the concrete floor at the feeding area was covered with rubber mats, while the remaining floors were not. In all three sections the manure was removed by alley scrapes. When the cows were fetched for milking, the staff cleaned the cubicles.

3.1.1 Milking

Milking was performed in an AMR (DeLaval AMR™) twice daily starting at 5.30 am and 4.00 pm throughout the study. The cows were fetched for milking by group, starting with group K1 followed by K3 and K2. Group K2 was milked last as cows with high SCC and intra-mammary infections with *Staphylococcus aureus* were placed in that group. The standard settings before the start of the trial was 3.5 s and 30 s for WT and TT, respectively. When the cows had entered the rotary, two TPM (TPM1 and TPM2) performed teat cleaning and teat stimulation by attaching a wash cup. Each robot washed two teats with tepid water (17-20 C°) and at the same time stimulated, pre-milked and dried the teat before milking. The TPM1 washed the rear teats and the TPM2 washed the front teats. Both robots started cleaning the right teat. At the two following robot stations attachment of teat cups was performed in the same order as the teat wash. Before the cow left the rotary, the teats were sprayed with a disinfectant/conditioner by a fifth robot. The minimum time for one full rotation of the 24 bail AMR was 20 min.

3.2 Animals

Between 118 (study 2) and 166 (study 1) cows of the Swedish Holstein and Swedish Red breeds participated in the studies. In 2014, the average annual milk yield and bulk milk SCC was 10 200 kg energy corrected milk (ECM) per cow and 187 500 cells/ml, respectively. Some cows were introduced to a group or moved from a group during the two studies, following calving, drying-off or diseases. Consequently, the number of animals observed varied within and between the groups during the two studies.

3.3 Experimental designs

As mentioned above the studies were performed using a cross-over design, meaning that each experimental unit (cow groups K1, K2, K3) was exposed to all treatments (WT and TT), one at a time for one week each. Experimental settings were used Monday to Friday during the study periods. Pre-trial settings were used on Saturday and Sunday. At every teat hygiene assessment occasion the milking units were attached manually immediately after the assessment. At the other milking occasions, the units were attached automatically.

3.3.1 Study 1 - Effects of three different wash times on teat hygiene and milking parameters

In study 1, cow groups K1, K2 and K3 were exposed to three different WT (3.5 s, 5.5 s and 7.5 s) as explained in Table 1. Teat hygiene assessments were performed during morning milkings on Mondays, Wednesdays and Fridays every week for three weeks. Data on milking parameters were collected during the other milkings performed Monday to Friday.

Table 1. Cross-over design and number of animals included in each cow group (K1, K2, K3) in a study where three different cow groups were subjected to three different wash times, one week at the time, in an automatic milking rotary system. Hygiene assessments of the teats were done before teat cup attachment during morning milkings Monday, Wednesday and Friday of each week.

Week no	**Wash Times (seconds)**			**Day of teat hygiene assessment (morning milking)**		**Numbers of animals assessed[1]**			
	K1	**K3**	**K2**			**K1**	**K3**	**K2**	**Total**
46	3.5	5.5	7.5	*Monday*		46	64	50	160
				Wednesday		50	64	49	163
				Friday		50	61	49	160
47	5.5	7.5	3.5	*Monday*		50	63	50	163
				Wednesday		49	62	49	160
				Friday		51	62	44	157
48	7.5	3.5	5.5	*Monday*		54	63	45	162
				Wednesday		55	66	45	166
				Friday		52	68	45	165
49	3.5	5.5	7.5	*Monday*		55	64	38	157
				Wednesday		56	63	39	158
				Friday		-	-	-	-
						568	700	503	**1771**
					Total	472	572	404	**1448**

[1] Hygiene assessments on Monday and Wednesday week 46 were excluded due to technical problems and the study was repeated Monday and Wednesday during week 49.

3.3.2 Study 2a and 2b - Effects of two different time-out times on teat hygiene and milking parameters

Two studies were performed using cow groups K1 and K3. In the first study (study 2a; December 2014) the cow groups were exposed to two different TT (25 s and 30 s) when the WT was set at 7.5 s for both TT treatments as explained in Table 2. In the second study (study 2b; January 2015), the cow groups K1 and K3 were exposed to two different TT (25 s and 30 s) when the WT was set at 5.5 s as explained in Table 3. Teat hygiene assessments were performed at morning milkings on Mondays, Wednesdays and Fridays every week for two weeks. Data on milking parameters were collected during the other milkings performed Monday to Friday.

Table 2. Cross-over design and number of animals included in each cow group (K1, K3) in a study where two different cow groups were subjected to two different time-out times, one week at the time, in an automatic milking rotary system using the teat wash time 7.5 s. Hygiene assessments of the teats were done during morning milkings Monday, Wednesday and Friday of each week.

Week no	**Time-out times (seconds)**		**Day of teat hygiene assessment (morning milking)**		**Number of animals assessed[1]**		
	K1	**K3**			**K1**	**K3**	**Total**
50	25	30	*Monday*		56	64	120
			Wednesday		53	69	122
			Friday		56	64	120
51	30	25	*Monday*		61	66	118
			Wednesday		64	62	126
			Friday		62	64	126
				Total	352 115	389 133	741 **248**

[1] All hygiene assessments except Wednesday week 50 and Friday week 51 were excluded due to technical problems.

Table 3. Cross-over design and number of animals included in each cow group (K1, K3) in a study where two different cow groups were subjected to two different time-out times, one week at the time, in an automatic milking rotary system using the teat wash time 5.5 s. Hygiene assessments of the teats were done during morning milkings Monday, Wednesday and Friday of each week.

Week no	**Time-out times (seconds)**		**Day of teat hygiene assessment (morning milking)**		**Number of animals assessed**		
	K1	**K3**			**K1**	**K3**	**Total**
3	25	30	*Monday*		52	66	118
			Wednesday		54	64	188
			Friday		54	67	121
4	30	25	*Monday*		54	63	117
			Wednesday		53	63	116
			Friday		55	65	120
				Total	322	388	**710**

3.4 Teat hygiene assessment before milking

All hygiene assessments were performed by the same person. On Tuesdays weeks 46 and 50, 2014, and week 4, 2015, teat hygiene assessments were performed during morning milking before the cows entered the AMR. The hygienic status of the right front teat (RF) of each cow was visually assessed using a five-grade scale according to Hovinen (2009) (Figure 1). In order to see the teats properly a headlight was used. In study 1 (week 46), the numbers of cows assessed were 47 (K1), 48 (K2) and 62 (K3), i.e. a total of 157 cows. In study 2a (week 50), the number of cows assessed were 55 (K1) and 62 (K3), i.e. a total of 117 cows. In study 2b (week 4), the number of cows assessed were 56 (K1) and 64 (K3), i.e. a total of 120 cows.

3.5 Teat hygiene assessment after teat wash

All hygiene assessments were performed by the same person. After the teat preparations by TPM1 and TPM2, the hygienic status of the RF and right rear (RR) teats of each cow was visually assessed using a five-grade scale according to Hovinen (2009) (Figure 1). In addition, the hygiene of the RF teat was also assessed by wrapping a moistened cloth (DeLaval Wetcel^{TM} 600) around the teat and evaluating the cleanliness of the cloth using a five-grade scale according to Hovinen (2009) (Figure 2). After wrapping the cloth around the teat the cloth-covered teat was stroked once from the teat base to the apex. In order to see the teats properly a headlight was used.

Figure 1. The scoring system according to Hovinen (2009) used to assess the cleanliness of teats. Pictures from the left to the right: 0 = clean (no visible dirt), 1 = almost clean (< 10% of the area is dirty), 2 = slightly dirty (10-20% of the area is dirty), 3 = dirty (20-50% of the area is dirty), 4 =extremely dirty (>50% of the area is dirty).

Figure 2. The scoring system according to Hovinen (2009) used to assess the cleanliness of a moistened cloth after wrapping it around the teat. Pictures from the left to the right: 0 = clean (no visible dirt), 1 = almost clean, 2 = slightly dirty, 3 = dirty, 4 =extremely dirty.

3.6 Registration of milking parameters

Data on milking parameters was automatically registered in the computer system at the research station for every milking, and was collected after each study. Milking parameters included in the study were registrations of incomplete milking, attachment failure and kick-off, and information on milk yield, milking time and milk flow on cow-level. The definition of incomplete milking was when the milk yield of a cow was less than 50% of expected yield, provided that the expected yield was more than 1 kg or the total milk yield at that specific udder quarter was less than 3 kg. If a cow had milked more than her individual pre-programmed limit, the system could designate her as completely milked even though she had kicked off a teat cup. In the statistical analyses data from the following milkings were used, Monday evening, Tuesday morning and evening, Wednesday evening, Thursday morning and evening, and Friday evening every week during the studies. The morning milkings on Monday, Wednesday and Friday were excluded since the teat cups were manually attached at those milkings.

3.7 Statistical analyses

To investigate if hygiene scores before milking differed between the cow groups the chi2-test was used. To investigate associations between WT or TT and the hygiene scores (visual or cloth), multilevel mixed-effect ordered logistic regression models were used. An ordinal model was used as the hygiene scores ranged from 0 to 4, representing increasing amounts of dirt on the teats (ordinal scale). A mixed-effect model takes both random and fixed factors into account and in this case the random factor was the repeated measurements within cow. Adjusting for that measurements within a cow are related and more similar than measurements between cows. An identity covariance structure was used for the random effect. To investigate associations between WT or TT and milking parameters, multilevel mixed-effect logistic and multilevel mixed-effect linear regression models were used. The fixed factors week, day, milking (pm/am) and milking station were included in all models. The descriptive statistics were mainly done using Excel while all the statistical analyses were performed using STATA 13.1 (StataCorp LP, College Station, TX, USA).

3.7.1 Study 1 – Effects of wash time

In total, 1771 hygiene assessments on 172 cows were done during weeks 46-49. Before statistical calculations were performed, hygiene assessments from Monday to Wednesday week 46 were excluded. Cows with some type of additional comment, e.g. dry teats, teats probably not washed at all (133 observations; 7%), or cows that had been observed twice (23 observations) were excluded. Thus, 1281 hygiene assessments from 168 cows were included in the statistical analyses on RF teat cleanliness and cloth cleanliness, and 1274 hygiene assessments from 168 cows on RR teat cleanliness. Data about milking parameters was collected from 3283 milkings from 175 cows. Dry cows and cows with registered diseases were excluded from the statistical analyses.

3.7.2 Study 2a – Effects of time-out time at WT 7.5s

A total of 741 hygiene assessments on 128 cows were performed during week 50-51. Due to various technical problems all assessments except from Wednesday week 50 and Friday week 51 were excluded. In total 247 hygiene assessments were available, but 30 (12%) had comments on insufficient teat cleaning and were excluded. Thus, 217 hygiene assessments from 120 cows were included in the statistical analyses on teat cleanliness and cloth cleanliness. Data about milking parameters was collected from 1369 milkings from 126 cows. Dry cows, cows with registered diseases and cows with additional comments were excluded from the statistical analyses.

3.7.3 Study 2b – Effects of time-out time at WT 5.5s

In total, 710 hygiene assessments on 124 cows were done during week 3-4. Before statistical analyses were performed cows with additional comments (63 observations) and cows that had been observed twice (3 observations) were excluded. Thus, 644 hygiene assessments from 124 cows were included in the statistical evaluations of RF teat cleanliness and cloth cleanliness, and 637 hygiene assessments from 122 cows on RR teat cleanliness. Data about milking parameters was collected from 1559 milkings from 124 cows. Dry cows, cows with registered diseases and cows with additional comments were excluded from the statistical analyses.

4. Results

4.1 Study 1 - Effects of three different wash times on teat hygiene and milking parameters

4.1.1 Associations between wash time and teat hygiene scores after teat wash

Significant differences in hygiene scores between cow groups were not found (P=0.70). The results from the multivariable analysis (Table 4) showed that the probability of high hygiene scores, i.e. dirty teats, on RF and RR teats, and on the RF cloth was significantly ($P \leq 0.005$) lower after WT 5.5 s and WT 7.5 s compared to after WT 3.5 s. The probabilities of high hygiene scores for RF and RR teats were significantly lower ($P \leq 0.01$) after WT 7.5 s than after WT 5.5 s. A similar tendency (P=0.06) was observed for the RF cloth. The proportion of RF cloths scored clean or almost clean was numerically lower than the proportion of RF teats scored clean or almost clean after all WT, but was the highest after WT 7.5 s. The associations between WT and hygiene scores did not change if cow breed (Swedish Holstein (SH), Swedish Red (SR)), cow age, and days in milk (DIM) were included in the models (data not shown). Cows over 200 DIM had cleaner teats (RF: $P \leq 0.01$, RR: $P \leq 0.01$, RF cloth: $P \leq 0.01$)) than cows below 200 DIM. SH-cows were cleaner than SR-cows on RF teats ($P \leq 0.05$). Cow age did not influence any of the parameters.

Table 4. Results from multivariable mixed-effect ordered logistic regression analysis of associations between wash time in an automatic milking rotary system and hygiene scores, assessed after teat wash, of right front teat (1281 observations from 168 cows), right rear teat (1274 observations from 168 cows), and cloth wrapped around right front teat (1281 observations from 168 cows). The probability of high hygiene scores on RF and RR teats, and on the RF cloth was significantly (*P*≤0.005) lower after WT 5.5 s and WT 7.5 s compared to after WT 3.5 s. The probabilities of high hygiene scores for RF and RR teats were significantly lower (*P*≤0.01) after WT 7.5 s than after WT 5.5 s. A similar tendency (*P*=0.06) was observed for the RF cloth.

	Visual assessment of right front teat				**Visual assessment of right rear teat**				**Visual assessment of cloth**			
Variable	*β*	S.E. (*β*)	95% CI[1] (*β*)	*P*-value	*β*	S.E. (*β*)	95% CI (*β*)	*P*-value	*β*	S.E. (*β*)	95% CI (*β*)	*P*-value
Wash time												
3.5s	Ref.[2]				Ref.				Ref.			
5.5s	-0.45	0.13	-0.70; -0.19	0.001	-0.36	0.13	-0.60; -0.11	0.005	-0.51	0.13	-0.77; -0.25	<0.001
7.5s	-0.78	0.13	-1.04; -0.52	<0.001	-0.80	0.13	-1.06; -0.55	<0.001	-0.77	0.13	-1.03; -0.50	<0.001
Week[3]												
1	Ref.				Ref.				Ref.			
2	0.60	0.20	0.20; 0.99	0.003	0.42	0.20	0.03; 0.80	0.03	0.53	0.20	0.14; 0.93	0.008
3	0.55	0.20	0.15; 0.95	0.007	0.42	0.20	0.03; 0.81	0.04	0.45	0.20	0.05; 0.85	0.028
4	0.44	0.24	-0.04; 0.91	0.07	0.98	0.24	-0.52; 1.45	<0.001	0.88	0.25	0.40; 1.36	<0.000
Day[4]												
1	Ref.				Ref.				Ref.			
3	0.29	0.13	0.03; 0.54	0.03	0.07	0.13	0.18; 0.32	0.59	0.34	0.13	0.08; 0.60	0.011
5	0.14	0.15	-0.15; 0.44	0.35	0.54	0.15	0.24; 0.83	<0.001	0.41	0.15	0.11; 0.72	0.007
Milking station												
1	Ref.				Ref.				Ref.			
2	0.65	0.35	-0.04; 1.34	0.06	0.13	0.34	-0.54; 0.80	0.71	0.55	0.35	-0.14; 1.25	0.12
3	0.20	0.35	-0.49; 0.90	0.56	0.69	0.35	0.01; 1.36	0.05	0.35	0.36	-0.37; 1.06	0.34
4	-0.19	0.36	-0.89; 0.52	0.60	0.57	0.34	-0.10; 1.24	0.09	0.30	0.36	-0.40; 1.00	0.40
5	-0.0005	0.37	-0.72; 0.72	1.00	0.26	0.35	-0.43; 0.95	0.45	0.13	0.36	-0.57; 0.83	0.71
6	-0.17	0.41	-0.97; 0.63	0.68	0.02	0.41	-0.78; 0.82	0.96	0.30	0.41	-0.51; 1.11	0.46
7	0.29	0.36	-0.41; 0.99	0.42	0.32	0.36	-0.39; 1.03	0.38	0.81	0.36	0.10; 1.51	0.03
8	.	.	.	.	.	.	.	.	.	.	.	.
9	0.13	0.36	-0.58; 0.85	0.71	0.29	0.35	-0.40; 0.97	0.42	0.41	0.37	-0.30; 1.13	0.26
10	0.25	0.37	-0.48; 0.97	0.50	0.51	0.36	-0.20; 1.21	0.16	0.59	0.37	-0.13; 1.30	0.11
11	0.26	0.36	-0.44; 0.98	0.46	0.55	0.35	-0.14; 1.23	0.12	0.32	0.37	-0.39; 1.04	0.38
12	-0.24	0.45	-1.13; 0.65	0.59	-0.07	0.45	-0.96; 0.81	0.87	0.33	0.45	-0.56; 1.21	0.47
13	0.22	0.42	-0.60; 1.05	0.60	0.26	0.41	-0.53; 1.06	0.52	0.13	0.42	-0.69; 0.95	0.76
14	0.07	0.37	-0.64; 0.79	0.84	0.61	0.35	-0.09; 1.30	0.09	0.40	0.36	-0.32; 1.11	0.28
15	-0.003	0.39	-0.77; 0.77	0.99	0.48	0.37	-0.24; 1.20	0.19	0.06	0.39	-0.70; 0.82	0.88
16	-0.37	0.38	-1.11; 0.37	0.32	0.06	0.35	-0.63; 0.76	0.86	-0.14	0.37	-0.87; 0.59	0.71
17	0.37	0.38	-0.38; 1.12	0.33	0.37	0.38	-0.37; 1.10	0.33	0.42	0.38	-0.33; 1.17	0.27
18	0.23	0.38	-0.51; 0.97	0.54	0.70	0.37	-0.03; 1.43	0.06	0.67	0.38	-0.07; 1.40	0.08
19	0.05	0.35	-0.63; 0.74	0.88	0.31	0.34	-0.35; 0.97	0.36	0.06	0.35	-0.62; 0.74	0.86
20	0.17	0.35	-0.53; 0.86	0.64	0.25	0.34	-0.42; 0.92	0.47	0.17	0.35	-0.52; 0.86	0.63
21	0.46	0.36	-0.24; 1.16	0.19	0.22	0.34	-0.45; 0.89	0.51	0.33	0.36	-0.37; 1.03	0.36
22	0.17	0.35	-0.52; 0.86	0.63	0.35	0.35	-0.33; 1.04	0.31	0.38	0.36	-0.33; 1.08	0.29
23	0.25	0.35	-0.44; 0.94	0.48	-0.11	0.34	-0.77; 0.56	0.75	0.44	0.35	-0.25; 1.13	0.21
24	0.21	0.36	-0.50; 0.92	0.56	0.45	0.34	-0.22; 1.11	0.19	0.35	0.36	-0.36; 1.05	0.34

[1]CI = confidence interval
[2]Ref = reference category
[3]Week; 1= calendar week no 46, 2 = calendar week no 47, 3 = calendar week no 48, 4 = calendar week no 49
[4]Day; 1 = Monday, 2 = Wednesday, 3 = Friday

4.1.2 Associations between wash time and milking parameters

4.1.2.1 Incomplete milking

The distribution of incomplete milkings per treatment is presented in Table 5. The results from the multivariable analysis showed that the probability that the cows had incomplete milking was significantly lower after WT 5.5 s and WT 7.5 s than after WT 3.5 s ($P \leq 0.001$). The difference between WT 5.5 s and WT 7.5 s was not significant ($P=0.35$). Including the whole material, a total of 470 registrations of individual udder quarters having incomplete milking were found. The distribution of those cases among quarters was 19% and 18% for RF and LF, respectively, and 30% and 33% for RR and LR, respectively. Cow breed, age and DIM did not have any significant influence on the results (data not shown).

4.1.2.2 Attachment failure

The distribution of attachment failures per treatment is presented in Table 5. The results from the multivariable analysis showed that the probability that the attachment units did not find the teats was significantly lower after WT 7.5 s than after WT 3.5 s ($P \leq 0.01$). The differences between WT 5.5 s and WT 3.5 s ($P=0.08$), and between WT 5.5 s and WT 7.5 s ($P=0.49$) were not significant. Including the whole material, a total of 394 registrations of individual quarters having attachment failure were found. The distribution of those cases among quarters was 28% and 38% for RF and LF, respectively, and 8% and 25% for RR and LR, respectively. Cow breed, age and DIM did not have any significant influence on the results (data not shown).

4.1.2.3 Kick-off

The distribution of kick-offs per treatment is presented in Table 5. The results from the multivariable analysis showed no significant differences between the treatments ($P>0.30$). Including the whole material, a total of 2477 registrations of individual quarters having kick-offs were found. The distribution of those cases among quarters was 14% and 11% for RF and LF, respectively, and 42% and 32% for RR and LR, respectively. Cow breed, age and DIM did not have any significant influence on the effect of WT (data not shown). Cows in early stages of lactation ($P \leq 0.05$) and younger cows ($P \leq 0.002$) had significantly more kick-offs than cows in late stages of lactation and older cows, respectively (data not shown).

Table 5. The distribution of numbers of milkings without and with registrations of incomplete milking, attachment failure and kick-off, for cows exposed to three different wash times in an automatic milking rotary system. The probability that the cows had incomplete milking was significantly lower after WT 5.5 s and WT 7.5 s than after WT 3.5 s ($P \leq 0.001$). The probability that the attachment units did not find the teats was significantly lower after WT 7.5 s than after WT 3.5 s ($P \leq 0.01$).

Remark	**Category**	**3.5 s n (%)**	**5.5 s n (%)**	**7.5 s n (%)**
Incomplete milking				
	No	1048 (92.6)	989 (96.4)	1084 (96.4)
	Yes	84 (7.4)	37 (3.6)	41 (3.6)
Attachment failure				
	No	1058 (93.5)	976 (95.1)	1076 (95.6)
	Yes	74 (6.5)	50 (4.9)	49 (4.4)
Kick-off				
	No	954 (84.3)	862 (84.0)	953 (84.7)
	Yes	178 (15.7)	164 (16.0)	172 (15.3)

4.1.2.4 Milk yield, milking time and milk flow

When including all cows, the average milk production, milking time and milk flow was 14.3 ± 5.4, 7.6 ± 3.4 minutes and 2.1 ± 0.8 kg/minute respectively. The average milk yield, milking time and milk flow per WT is presented in Table 6. Results from the multivariable analysis showed that the milk flow was significantly higher after WT 5.5 s and WT 7.5 s than after WT 3.5 s ($P \leq 0.05$). The difference in milk flow between WT 5.5 s and WT 7.5 s ($P=0.67$) was not significant. However, the difference between WT 3.5 s and WT 7.5 s was 0.8 kg of milk yield, which corresponds to 5% of the total milk yield. Cow breed, age and DIM had significant associations with milk flow (SH higher than SR ($P \leq 0.01$) higher in early lactation ($P \leq 0.05$), older cows higher than young cows ($P \leq 0.05$) but did not influence the associations between WT and milk flow (data not shown)).

Table 6. The average milk yield, milking time and milk flow for cows exposed to three different wash times in an automatic milking rotary system (n=3281 observations from 175 cows). Milk flow was significantly higher after WT 5.5 s and WT 7.5 s than after WT 3.5 s ($P \leq 0.05$).

Variable	**3.5 s mean (SD)**	**5.5 s mean (SD)**	**7.5 s mean (SD)**
Milk yield (kg)	13.9 (5.6)	14.4 (5.4)	14.7 (5.6)
Milking time (min)	6.9 (1.5)	7.0 (1.5)	7.1 (1.4)
Milk flow (kg/min)	2.0 (0.9)	2.1 (0.9)	2.1 (0.8)

4.2 Study 2a – Effects of two different time-out times (WT 7.5 s) on teat hygiene and milking parameters

4.2.1 Association between time-out time and teat hygiene scores after teat wash

The distribution of hygiene score of RF teats before teat wash did not differ significantly between cow groups K1 and K3 (*P*=0.87). The results from the multivariable analysis showed that the hygiene scores did not differ significantly between TT 25 s and TT 30 s (RF: *P*=0.45; RF cloth *P*=0.49; RR: *P*=0.56). The proportion of clean or almost clean assessments of the RF teats was overall lower when using the cloth than when using the visual assessment of the teat. As the number of hygiene assessments was low those results are only presented in text.

4.2.2 Associations between time-out time and milking parameters

4.2.2.1 Incomplete milking

The distribution of incomplete milkings per TT treatment is presented in Table 7. The results from the multivariable analysis showed no significant difference between treatments (*P*=0.70).

4.2.2.2 Attachment failure

The distribution of attachment failures per TT treatment is presented in Table 7. The results from the multivariable analysis showed no significant difference between treatments (*P*=0.81).

4.2.2.3 Kick-off

The distribution of kick-offs per TT treatment is presented in Table 7. The result from the multivariable analysis showed no significant difference between treatments (*P*=0.22).

Table 7: The distribution of numbers of milkings without and with registrations of incomplete milkings, attachment failures and kick-offs, for cows exposed to two different time-out times using the wash time 7.5 s in an automatic milking rotary system. There were no significant differences between treatments on either incomplete milkings (*P*=0.70), attachment failures (*P*=0.81) or kick offs (*P*=0.22).

Variable	**Category**	**30 s** **n (%)**	**25 s** **n (%)**
Incomplete milking			
	No	675 (95.5)	631 (95.3)
	Yes	32 (4.5)	31 (4.7)
Attachment failure			
	No	680 (96.2)	634 (95.8)
	Yes	27 (3.8)	28 (4.2)
Kick-off			
	No	609 (86.1)	582 (87.9)
	Yes	98 (13.9)	80 (12.1)

4.2.2.4 Milk yield, milking time and milk flow

When including all cows, the average milk production, milk time and milk flow were 15.8 ± 5.3 kg, 7.8 ± 3.1 minutes and 2.2 ± 0.8 kg/minute respectively. The average milk yield, milking time and milk flow per TT treatment is presented in Table 8. The milk flow was significantly lower in TT 25 s than in TT 30 s ($P \leq 0.05$).

Table 8. The average milk yield, milking time and milk flow for cows exposed to two different time-out times using the wash time 7.5 s in an automatic rotary system (n=1551 observations from 124 cows). The milk flow was significantly lower in TT 25 s than in TT 30 s ($P \leq 0.05$).

Variable	**30 s mean (SD)**	**25 s mean (SD)**
Milk yield (kg)	16.1 (5.2)	15.5 (5.4)
Milking time (min)	7.7 (3.0)	7.8 (3.3)
Milk flow (kg/min)	2.3 (0.8)	2.2 (0.8)

4.3 Study 2b – Effects of two different time-out times (WT 5.5 s) on teat hygiene and milking parameters

4.3.1 Association between time-out time and teat hygiene scores after teat wash

Significant differences in hygiene scores between cow groups were not found (P=0.31). The results from the multivariable analysis (Table 9) showed that the hygiene scores did not differ significantly between TT 25 s and TT 30 s ($P>0.05$). However, the visual RF hygiene score tended to be higher after TT 25 s than after TT 30 s (P=0.095). The proportion of clean or almost clean assessments of the RF teats was overall lower when using the cloth than when using the visual assessment of the teat. The associations between TT and hygiene scores did not change if cow breed, cow age, and DIM were included in the models (data not shown). Hygiene scores were not significantly affected by breed, age and DIM (data not shown).

Table 9. Results from multivariable mixed-effect ordered logistic regression analysis of associations between time-out time using the wash time 5.5 s in an automatic milking rotary system and hygiene scores, assessed after washing and with different time-out times, of right front teat (644 observations from 124 cows), right rear teat (637 observations from 122 cows), and cloth wrapped around right front teat (644 observations from 124 cows), respectively. The hygiene scores did not differ significantly between TT 25 s and TT 30 s ($P>0.05$). However, the visual RF hygiene score tended to be higher after TT 25 s than after TT 30 s ($P=0.095$).

	Visual assessment of right front teat				**Visual assessment of right rear teat**				**Visual assessment of cloth**			
Variable	***β***	**S.E. (*β*)**	**95% CI[1] (*β*)**	***P*-value**	***β***	**S.E. (*β*)**	**95% CI (*β*)**	***P*-value**	***β***	**S.E. (*β*)**	**95% CI (*β*)**	***P*-value**
Time-out time												
30s	Ref.[2]				Ref.				Ref.			
25s	0.26	0.16	-0.04; 0.56	0.095	0.20	0.15	-0.10; -0.50	0.18	0.15	0.16	-0.15; -0.46	0.33
Week[3]												
1	Ref.				Ref.				Ref.			
2	0.46	0.16	0.15; 0.76	0.003	0.34	0.15	0.04; 0.64	0.03	0.12	0.16	-0.19; 0.43	0.46
Day[4]												
1	Ref.				Ref.				Ref.			
3	0.15	0.19	-0.22; 0.52	0.43	-0.11	0.19	-0.48; 0.25	0.55	0.07	0.20	-0.31; 0.45	0.72
5	0.10	0.19	-0.27; 0.46	0.59	-0.05	0.18	-0.41; 0.31	0.78	-0.05	0.19	-0.43; 0.32	0.77
Milking station												
1	Ref.				Ref.				Ref.			
2	-0.40	0.60	-1.56; 0.77	0.50	-0.94	0.59	-0.21; 2.09	0.11	-0.27	0.60	-1.46; 0.91	0.65
3	0.57	0.55	-0.51; 1.65	0.30	1.63	0.53	0.59; 2.67	0.002	0.36	0.55	-0.71; 1.43	0.51
4	0.25	0.55	-0.82; 1.32	0.64	0.92	0.54	-0.13; 1.98	0.09	0.51	0.55	-0.57; 1.59	0.36
5	-0.37	0.54	-1.43; 0.68	0.49	0.53	0.53	-0.51; 1.57	0.32	-0.07	0.54	-1.13; 0.99	0.90
6	0.39	0.54	-0.67; 1.46	0.47	0.41	0.53	-0.63; 1.45	0.44	0.22	0.55	-0.86; 1.29	0.69
7	0.12	0.54	-0.94; 1.19	0.82	0.78	0.54	-0.28; 1.83	0.15	-0.59	0.56	-1.69; 1.19	0.50
8	1.47	1.04	-0.57; 3.51	0.16	0.26	1.05	-1.79; 2.32	0.80	-0.46	1.15	-2.71; 1.79	0.69
9	0.05	0.57	-1.06; 1.17	0.92	0.87	0.55	-0.21; 1.95	0.11	0.07	0.59	-1.22; 1.08	0.90
10	0.57	0.54	-0.48; 1.62	0.29	0.58	0.53	-0.46; 1.62	0.28	0.65	0.56	-0.45; 1.74	0.25
11	-0.78	0.54	-1.83; 0.27	0.15	0.11	0.53	-0.94; 1.15	0.84	-0.17	0.54	-1.23; 0.90	0.76
12	-0.29	0.56	-1.39; 0.81	0.60	0.37	0.55	-0.71; 1.44	0.51	-0.35	0.58	-1.49; 0.79	0.55
13	0.49	0.55	-0.58; 1.56	0.37	0.73	0.53	-0.31; 1.78	0.17	-0.11	0.56	-1.20; 0.97	0.84
14	-0.06	0.55	-1.13; 1.02	0.92	0.70	0.54	-0.35; 1.76	0.19	-0.11	0.56	-1.21; 0.99	0.85
15	-0.05	0.54	-1.11; 1.01	0.92	0.51	0.52	-0.51; 1.54	0.33	-0.25	0.55	-1.34; 0.83	0.65
16	-0.29	0.56	-1.38; 0.80	0.60	0.55	0.53	-0.49; 0.60	0.30	0.42	0.56	-1.68; 1.52	0.46
17	0.75	0.56	-0.35; 1.84	0.18	0.31	0.54	-0.75; 1.37	0.57	0.48	0.57	-0.64; 1.60	0.40
18	0.78	0.61	-1.97; 0.42	0.20	0.75	0.60	-0.43; 1.92	0.21	0.08	0.61	-1.11; 1.27	0.90
19	0.20	0.55	-0.87; 1.28	0.71	0.96	0.52	-0.06; 1.99	0.07	0.46	0.56	-0.63; 1.56	0.41
20	0.61	0.56	-1.71; 0.49	0.28	0.54	0.54	-0.51; 0.59	0.31	0.07	0.55	-0.99; 1.15	0.89
21	-0.36	0.52	-1.39; 0.66	0.49	-0.29	0.52	-1.31; 0.73	0.58	0.29	0.53	-0.75; 1.33	0.59
22	-0.09	0.55	-1.16; 0.99	0.87	0.55	0.53	-0.50; 1.60	0.30	0.29	0.56	-0.81; 1.38	0.61
23	0.24	0.52	-0.77; 1.25	0.64	0.89	0.51	-0.10; 1.89	0.08	0.30	0.53	-0.73; 1.33	0.57
24	-0.33	0.54	-1.38; 0.72	0.54	-0.98	0.53	-1.13; 0.93	0.85	0.25	0.53	-0.79; 1.30	0.64

[1]CI = confidence interval

[2]Ref = reference category

[3]Week; 1= calendar week no 3, 2 = calendar week no 4

[4]Day; 1 = Monday, 2 = Wednesday, 3 = Friday

4.3.2 Associations between time-out time and milking parameters

4.3.2.1 Incomplete milking

The distribution of incomplete milkings per TT treatment is presented in Table 10. The results from the multivariable analysis showed that the difference between treatments was not significant (*P*=0.82). The associations between TT and hygiene scores did not change if cow breed, cow age, and DIM were included in the models (data not shown). Cow breed, age and DIM did not have any significant influence on the results (data not shown).

4.3.2.2 Attachment failure

The distribution of attachment failures per TT treatment is presented in Table 10. The results from the multivariable analysis showed that the difference between treatments was not significant (*P*=0.87). Cow breed, age and DIM did not have any significant influence on the results (data not shown).

4.3.2.3 Kick-off

The distribution of kick-offs per TT treatment is presented in Table 10. The result from the multivariable analysis showed that the number of registrations of kick-off was significantly lower after TT 30 s than after TT 25 s ($P \leq 0.05$). Cow breed, age and DIM did not have any significant influence on the results (data not shown).

Table 10: The distribution of numbers of milkings without and with registrations of incomplete milkings, attachment failures and kick-offs, for cows exposed to two different time-out times in an automatic milking rotary system. Number of registrations of kick-off was significantly lower after TT 30 s than after TT 25 s ($P \leq 0.05$).

Variable	**Category**	**30 s** **n (%)**	**25 s** **n (%)**
Incomplete milking			
	No	747 (97.0)	767 (97.2)
	Yes	23 (3.0)	22 (2.8)
Attachment failure			
	No	752 (97.7)	772 (97.9)
	Yes	18 (2.3)	17 (2.2)
Kick-off			
	No	675 (87.7)	674 (85.4)
	Yes	95 (12.3)	115 (14.6)

4.3.2.4 Milk yield, milking time and milk flow

When including all cows, the average milk production, milk time and milk flow were 16.9 ± 5.0 kg, 8.0 ± 3.0 minutes and 2.3 ± 0.8 kg/minute respectively. The average milk yield, milking time and milk flow per TT treatment is presented in Table 11. The difference in milk flow between TT 30 s and TT 25 s was not significant (*P*=0.15). Cow breed, age and DIM did not have any significant influence on the effect of TT (data not shown). The milk flow was significantly higher in SH-cows than in SR-cows (*P*=0.008), but age and DIM did not have a significant effect on the milk flow (data not shown).

Table 11. The average milk yield, milking time and milk flow for cows exposed to two different time-out times in an automatic rotary system (n=1551 observations from 124 cows). The difference in milk flow between TT 30 s and TT 25 s was not significant (*P*=0.15).

Variable	**30 s mean (SD)**	**25 s mean (SD)**
Milk yield (kg)	16.8 (5.3)	17.0 (4.8)
Milking time (min)	7.5 (1.4)	7.5 (1.4)
Milk flow (kg/min)	2.3 (0.8)	2.3 (0.8)

5. Discussion

5.1 Study 1 - Effects of wash time on teat hygiene and milking parameters

In the present study it was found that longer WT improved the teat hygiene, which was in line with the hypothesis. Therefore, the cleanest teats were found after WT 7.5 s. To my knowledge, studies on how different WT in AMR or other AMS affect the teat hygiene have not been published.

It was not possible to study the left side of the udder in this study, or to do a whole udder hygiene assessment, due to limited time and because of the design of the rotary. Given the fact that each TPM always starts with the right front or rear teat followed by the left front or rear teat, effects on the hygiene of the left teats would have gone unnoticed. In order to overcome this problem in future studies it may be suitable to use the cloth test on the left front or left rear teat instead of on the right teats. In the original project plan of this study the intention was to measure the bacterial counts in composite milk samples of individual cows. Unfortunately, those samples were omitted since the equipment for collecting composite milk samples was difficult to clean between milkings and the samples were, therefore, contaminated. Milk that was delivered to the dairy plant from the research station farm during the study, had, however, no registered complaints on high bacterial counts. Measurement of total bacterial count in milk would have been an objective and indirect measurement on udder hygiene, which would have been a relevant complement in order to evaluate milking hygiene.

In the study, it was also found that WT had significant effects on milking parameters. The risk of attachment failure and incomplete milking was higher, and the milk flow was lower after WT 3.5 s compared to WT 5.5 s and WT 7.5 s. However, there were no significant differences between WT 5.5 s and WT 7.5 s.

When a cow is registered as incompletely milked, she will be redirected to the rotary platform in order to be "remilked". If a large amount of incomplete milkings occurs during milking, the milking efficiency of the AMR will be reduced. It will also reduce the milk production of the individual cow/quarter on short term (Lakic et al., 2009, 2011, Kolbach et al., 2013, Ljunggren 2015).

To my knowledge, there are no previous studies on how incomplete milking is affected by teat wash. However, Kolbach et al. (2013) found that use of WT 3.5 s resulted in higher attachment success and faster attachment compared to no teat wash, in a prototype robotic rotary. It has been observed that attachment failure may be due to teat conformation (Miller et al., 1995; Ljunggren 2015), but why teat hygiene was associated with attachment success is not clear. A reasonable explanation may be that it is easier for the camera to identify the teat location if the teat is clean. Previous studies have found that attachment failure may result in decreased milk production, on short term (Bach and Busto, 2005), reduced well-being of the cow (Stefanowska et al., 2000), and increased risk of milk leakage (Stefanowska et al., 2000, Persson Waller et al., 2003), which may increase the risk for intra-mammary infections and mastitis (Waage et al., 1998).

No studies on how different WT in AMR™ or VMS™ affect the milk flow have been found. When Kolbach et al. (2013) studied the effects of teat wash (WT 3.5 s) compared to no teat wash on average milk flow, no significant differences were found. However, when the milking interval was ≤8 hours the wash treatment gave higher peak flow rates than the control group. Additionally, Davis et al. (2008) found a higher quarter milk flow when using teat wash (WT 5.5 s) compared to no teat wash in a VMS™. It was also found that teat wash resulted in overall longer crate time and lower harvest rate compared to no teat wash.

5.2 Study 2 - Effects of time-out time, while using wash time 5.5 s or 7.5 s, on teat hygiene and milking parameters

To my knowledge, studies on how different TT affect teat hygiene and milking parameters have not been performed previously.

In the present study two different TT, 25 s and 30 s, were tested using WT 5.5 s and WT 7.5 s. It was found that there were no significant differences in either hygiene assessments, incomplete milkings or attachment failures between TT in the two studies. As mentioned above the teats on the right side of the udder is cleaned first by each TPM. This means that there is a higher risk that the teats on the left side are missed during washing. Hence, the results on the hygiene assessment should be interpreted with caution since the evaluation of the teat hygiene was only made on the right side. The number of hygiene assessments included in the statistical evaluation from the study using WT 7.5 s was low due to technical problems, which makes the results uncertain. The results on attachment failure and incomplete milking are more reliable since they were based on a larger number of observations.

More kick-offs were registered when using TT 25 s compared to when using TT 30 s when the WT was 5.5 s, but this difference was not seen when the WT was 7.5 s. It is not clear why these results were found and further studies are therefore needed in order to evaluate the reasons. Kick-offs is most likely a result of factors like stress, pain and drop in teat cup vacuum level.

Furthermore, a lower milk flow (mainly due to lower milk yield) was found when using TT 25 s compared to TT 30 s when the WT was 7.5 s, but not when using WT 5.5 s. The reasons for this finding are not clear and need to be further investigated.

5.3 Additional comments

In the present study it was found that the hygiene score of the right front teat was higher when using the cloth compared to when using the visual evaluation of the teat. The relative findings of the two methods were in line with Hovinen (2009). It is logical that more dirty teats were found when using the cloth method since the method makes it possible to include the whole teat in the hygiene evaluation. In comparison, the visual method only assesses the lateral part of the teat. Moreover, it is more likely that a moisture cloth finds dirt that the eye easily may miss. Both methods are subjective but the cloth method is considered to give a more true evaluation of the teat hygiene. Another factor that needs to be considered is that the person who performed the practical experiments was aware of the WT and/or TT tested, which might have affected the hygiene assessments.

6. Conclusions

The teat WT before milking cannot be shortened from default 5.5 s to 3.5 s (default TT 30 s) without risk for deterioration of the teat hygiene and milking parameters studied. The best hygiene score was found after using WT 7.5 s.

The TT used at teat cleaning can probably be shortened from 30 s to 25 s at WT 5.5 s without deterioration of the teat hygiene and milking parameters studied. However, additional assessment of the effects on teat hygiene on the left side of the udder is needed before a recommendation can be given.

7. Suggestions for further studies

As only the right front and right rear teats were assessed during the present study, the hygiene evaluation was not optimal. Therefore, it may be advisable to repeat some of the studies. Given the experience from the present project it is suggested that the cloth test is used on the right front and left front teats, or if possible on all four teats, in further studies. The inclusion of other hygiene measurement tools should also be considered, such as evaluation of the hygiene of the teat apex.

The present study indicates that the proportion of kick-offs differed between TT 25 s and TT 30 s when using WT 5.5 s. As it is not known why this difference was found, a study focusing on this problem may be worthwhile.

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