Effectiveness of automatic cleaning of udder and teats and effects of hygiene management

Report on hygiene measures resulting in adequate teat cleaning

February 2004

Information

This report is produced within the EU project *Implications of the introduction of automatic milking on dairy farms* (QLK5 -2000-31006) as part of the EU-program 'Quality of Life and Management of Living resources'.

Liability

Availability

This report is freely available for every person interested at:
http://www.automaticmilking.nl

Effectiveness of automatic cleaning of udder and teats and effects of hygiene management

Report on hygiene measures resulting in adequate teat cleaning

Dr. Karin Knappstein
Nele Roth
Dr. Hans-Georg Walte
Dr. Joachim Reichmuth
Institute for Hygiene and Food Safety
Federal Research Centre for Nutrition and Food
Kiel, Germany

February 2004
Deliverable D15

Correspondence:
Karin Knappstein
Hermann Weigmann-Str. 1
24103 Kiel, Germany
knappstein@bafm.de

Abstract

During an investigation on teat cleaning efficiency of different Automatic Milking (AM) systems working in practice differences were detected not only between systems of different brands but also between farms working with the same AM system. In addition, teat contamination before cleaning was of significant influence on teat cleaning efficiency. Therefore the second part of the study focused on management factors to determine which measures are significantly associated with teat contamination.

The investigation was performed on 18 farms. Differential bacterial counts in bulk tank milk were determined to find potential failures in milk quality. High coliform counts on 8 farms indicated insufficient teat cleaning and/or failures in system cleaning. On 14 farms thermoduric bacteria exceeding 200 cfu/ml showed potential problems with system cleaning although TBC was well below 10000 cfu/ml, but thermoduric bacteria may also have originated from bedding material.

In fresh bedding material different levels of bacterial contamination were found, with lowest total bacterial counts in specially treated sawdust and in sand. Coliform bacteria on teat surfaces are not necessarily due to faecal contamination, because these bacteria were also found in fresh bedding.

The hygiene management on farms was evaluated by means of a questionnaire-based interview with the farmer. In addition, a checklist was used to determine the actual hygiene status of certain areas on the farm. An analysis of variance was performed including 45 questions of the questionnaire and 17 aspects of the hygiene checklist.

AM specific management factors associated with high average teat contamination on farms were: replacement of teat cleaning device $\leq$ once per year, moderate/poor status of the teat cleaning device, average milking frequency per day $\leq$ 2.5 and no selection of cows for robot acceptance ($p<0.10$). Factors not directly related to AM involved contamination of cubicles: less than one cubicle per cow, cows lying on alleys present in the herd, addition of fresh bedding material less than once per day, no selection of cows for udder health, moderate/poor status of bedding material and moderate/poor status of claws were significantly related to high teat contamination.

Additional factors like the general impression of the robot, cleaning frequency of the milking box, status of teat cups and the use of cow brushes in the barn were probably more closely related to the general attitude of the farmer towards hygiene than to teat cleanliness.

Other factors can be regarded as generally accepted hygiene measures, because nearly all farmers practised cubicle cleaning twice per day, shearing of udders and cutting of tail heads. These factors should therefore be considered as basic hygiene measures.

Although the cause and effect relationship between parameters used to evaluate hygiene management on farms and teat cleanliness was not always very strong these factors should be considered when improvements of teat cleanliness are intended. Even with very good conditions it will be unavoidable that individual cows will have soiled teats, but farm management should aim for clean udders in the majority of cows. Apart from those factors which are specific for AM systems (e.g. frequency of replacing teat cleaning devices, milking frequency) it can be expected that the factors mentioned above can also improve teat cleanliness on farms with conventional milking systems.

Table of contents

1 Introduction

High quality of raw milk is a prerequisite for production of high quality milk products. In Europe a threshold of 100 000 colony forming units (cfu)/ml for total bacterial count (TBC) is fixed for bulk tank milk at delivery by Council Directive 92/46/EEC. In practice, much lower TBC are achievable. When all aspects of hygiene are regarded TBC of 10000 cfu/ml should not be exceeded (Reinemann, 1997).

Teat and udder surfaces belong to the three main sources for bacterial contamination of raw milk. Other main sources are mastitis pathogens and bacteria from milk contact surfaces (Slaghuis, 1996, Sumner, 1996). When teats are not sufficiently cleaned before milking milk of individual cows can yield high bacterial counts. Therefore effective teat cleaning before milking is important to avoid bacterial contamination of milk. Clean teats before milking are also an explicit demand according to Council Directive 89/362/EEC.

In Automatic Milking (AM) systems teat cleaning procedures are mechanized. Usually the cleaning intensity is determined for the whole herd based on cleaning time or number of brush movements. In some systems it is possible to apply more intensive teat cleaning for individual cows. Until now no online control mechanisms are available to check for success of the teat cleaning procedure.

In the first part of this investigation teat cleaning efficiencies of the different AM systems installed in practice were evaluated on 18 farms working with AM systems of 6 different brands (Report D14). The analysis indicated that brand has a significant influence on teat cleaning efficiency. Significant differences of cleaning efficiency between the three farms working with the same AM system showed that also management on the farm is important for successful teat cleaning. These conclusions were underlined by a significant influence of initial teat contamination on teat cleaning efficiency.

It was assumed from the beginning that the mechanized systems would not be able to deal with very dirty teats. Even if a control system for the teat cleaning procedure was available, more time would be spent on cleaning of very dirty teats and restricting the available time for milking in the AM system. In addition, teats from well bedded cows are less contaminated than teats from cows kept on minimal bedding when the same cleaning method is applied (Hansen, 1973). Therefore it is desirable that cows have teats as clean as possible when they enter the AM system.

During the investigation described above the hygiene management on the 18 farms was evaluated by means of a questionnaire and a checklist. Additional information was gained by determination of differential bacterial counts in bulk tank milk. An analysis of variance was carried out to find management factors significantly associated with teat cleanliness.

2 Materials and Methods

The investigation was performed on 18 farms working with 6 different brands of AM systems (3 farms per brand). The following AM brands were included: DeLaval, Fullwood, Insentec, Lely Industries, Prolion/Gascoigne Melotte, Westfalia Surge GmbH.
The addresses of farms were provided by the manufacturers. During a one-day visit the investigations on teat cleaning efficiency as described in Report D14 as well as of management factors were performed.

2.1 Teat contamination before cleaning

The procedure to determine teat contamination before cleaning was described in detail in Report D14. Briefly 50 cows were sampled on each farm. When fewer cows were available as many cows as possible were sampled during a period of approximately 8 hours. The cows were sampled after entering the AM system before start of the robot actions. From each cow the sampling was performed on two teats, one front and one hind teat of the side facing the investigator.
Sampling was performed with sterile cotton wool swabs soaked in 0.85 % NaCl, 0.1 % peptone. 3 strokes from each of two opposite sides of a teat were taken. After replacing the swab in 8.0 ml NaCl-peptone and vortexing for 10 seconds the swab was removed. About 1.5 ml of the solution was used for measurements of adenosine-tri-phosphate (ATP) on the farm using the system HyLite® 2 (Merck, 64293 Darmstadt, DE) for determination of ATP in fluids. The remaining solution was cooled for max. 24 h until further analysis. The determination of total bacterial counts (TBC) in the remaining swab solution was according to IDF Standard 100B:1991.
Results were Log_{10} transformed for further analysis and are presented in Log_{10} cfu/ml swab solution (TBC) and Log_{10} RLU (ATP).

2.2 Bacteriological quality of bulk tank milk

On each of 18 farms (3 farms per AM brand) a sample of bulk tank milk was taken before starting teat sampling. Milk was sampled with a sterile sampling vessel via the manhole of the tank after thoroughly mixing of bulk tank milk. The sampling time was independent from the collection interval of bulk milk. Milk samples were cooled until further analysis. Differential bacterial counts were determined within 24 hours according to standard procedures:

- Total bacterial count (TBC) – IDF (1991)
- Coliform bacteria (IDF, 1985)
- Thermoduric bacteria (Frank et al., 1985)

The limit of quantification was 10 cfu/ml. Lower bacterial counts were set to 10 cfu/ml for presentation of results.

2.3 Bacterial counts in fresh bedding

To determine the potential contribution of bacteria originating from bedding material to teat contamination a sample of fresh bedding material was taken from each farm. 5 g of sample were used for the analysis. The investigation of differential bacterial counts was according to standard procedures as described in chapter 2.2. The limit of quantification was 100 cfu/g.

2.4 Questionnaire on farm management

The evaluation of aspects of farm management was performed by means of a questionnaire based interview of 18 farmers (3 farms per AM brand). The questionnaire focused on aspects related to teat cleanliness. In addition some questions on general hygiene were included.
45 questions were included into the analysis. The questions belonged to the categories general housing, AM system, lying area, feeding area and cow management.

2.5 Checklist on hygiene status

A checklist on hygiene management was applied on all 18 farms. 17 different aspects regarding AM system, housing and cows were scored according to a system of 3 score levels:
score 1 = good
score 2 = moderate
score 3 = poor

A detailed explanation of scores is given in Annex I.
As scoring is a subjective means to determine levels of contamination and individuals have different ideas on cleanliness (Report D13) the scoring was performed by the same person on all farms to reduce variation.

2.6 Statistical analysis

2.6.1 Descriptive statistics

The results of analysis of teat contamination were summarized as means per farm regarding TBC and ATP respectively. These data were included into the analysis of variance. A more detailed description of teat contamination before cleaning is presented in Report D14.

Results of differential bacterial counts in bulk milk and in fresh bedding material are presented per farm and per bedding material.

Selected results of the questionnaire on farm management and of the hygiene checklist are presented in tables.

2.6.2 Analysis of variance

The effect of the different factors from the questionnaire and the hygiene checklist on teat contamination before teat cleaning was evaluated by an analysis of variance using the GLM (General Linear Model) model of SAS®, release 8.01 according to the following equation:

Y_{ij} = $\mu + factor_i + e_{ij}$

Y_{ij} = dependent variable mean teat contamination per farm before teat cleaning (for TBC: Log_{10} cfu/ml, for ATP: Log_{10} RLU)
μ = overall mean
$factor_i$ = effect of factor i (factor from a list of management factors based on questionnaire and checklist)
e_{ij} = random residual error

Factors were only included when at least three farms were observed with variation for this factor.
A detailed description of the variables and combination of values used for the analysis to achieve a more equal distribution is given in Annex II.
The low number of farms was associated with limited variability, therefore statistical significance was assumed at $p<0.10$ and no interactions between factors were tested in the model.

3 Results and Discussion

3.1 Teat contamination before cleaning

Large variation was seen for teat contamination before cleaning with ranges of 3 to 5 Log-units between minimum and maximum values for TBC and 2 to 4 Log-units for ATP on individual farms (Report D14). In figure 1 teat contamination per farm is summarized as means for TBC and ATP respectively.

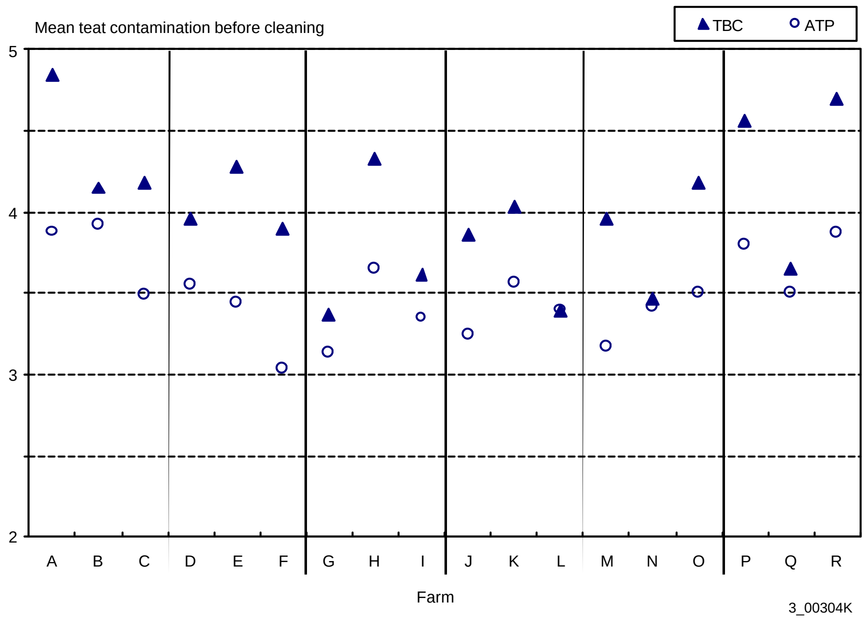

Figure 1: Mean teat contamination per farm before cleaning, determined by teat swabs (TBC: Log_{10} cfu/ml; ATP: Log_{10} RLU)

The correlation between TBC and ATP was r=0.72 for teat swabs taken from individual teats before cleaning. The relationship between TBC and ATP varied between individual farms. Therefore the analysis of variance was performed on both parameters separately (chapter 3.6).

3.2 Bacteriological quality of bulk tank milk

If milking is performed under good hygienic conditions coliform counts in bulk tank milk should not exceed 100 cfu/ml. Coliform counts exceeding 1000 cfu/ml are an indication for growth at milk contact surfaces due to insufficient cleaning of milking equipment (Reinemann, 1997).
On 8 farms milking with AM systems of 5 brands coliform counts in bulk tank milk exceeding 100 or 1000 cfu/ml resp. indicated insufficient teat cleaning before milking and/or insufficient system cleaning (figure 2).

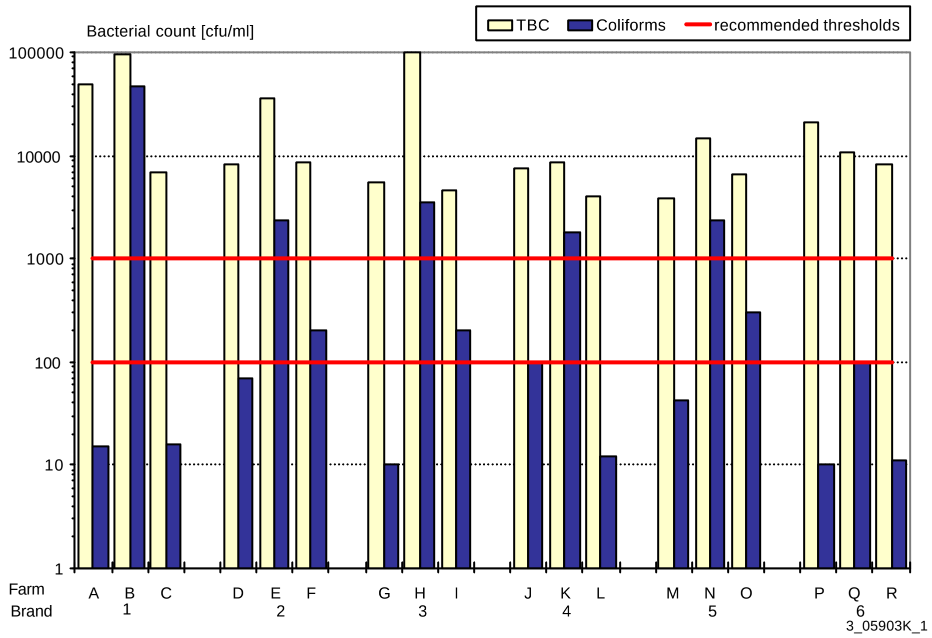

Figure 2: TBC and coliform counts in bulk tank milk

The extremely high coliform counts were also mainly responsible for the high TBC of bulk milk on farm B. The high counts of coliforms on this farm were due to very bad housing conditions with extremely dirty cows. The former barn for heifers was used for cows without adjustment of cubicles dimensions for the size of cows. The hygienic status of cubicles was very bad. Interesting is also that the farmer reported a high number of clinical cases of coliform mastitis. Although he attributed this problem only to purchased cows not accepting the cubicles, the mastitis cases were probably related to the bad housing conditions.

For thermoduric bacteria a threshold of 200 cfu/ml in bulk tank milk is recommended (Reinemann, 1997). Only on 4 farms contents of thermoduric bacteria in bulk tank milk were below the recommended threshold (figure 3). This is an indication of failures in system cleaning (Reinemann, 1997).

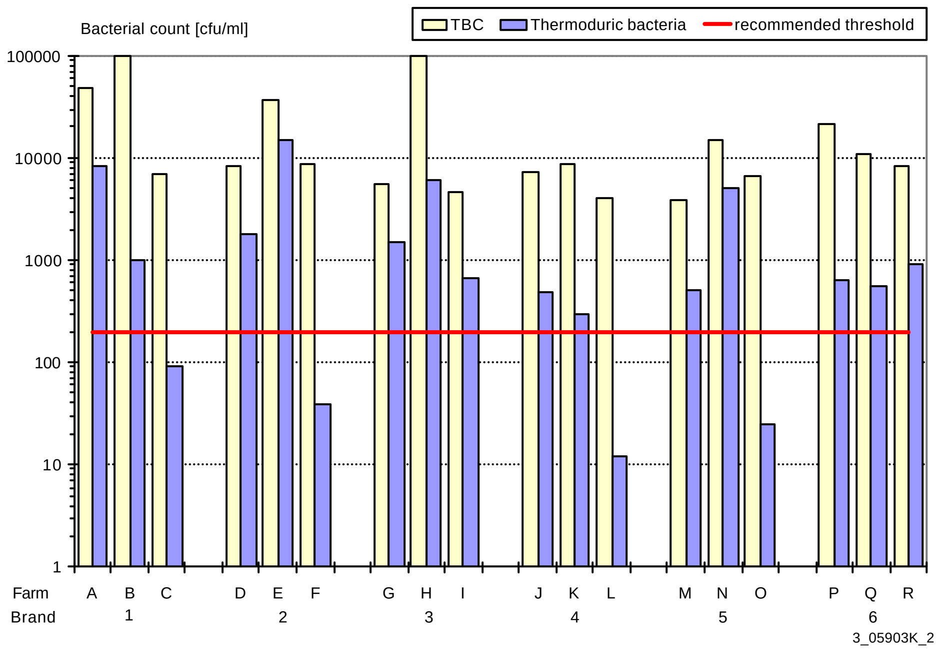

Figure 3: TBC and thermoduric bacteria in bulk tank milk

On 14 farms high counts of thermoduric bacteria were observed although TBC were below or close to 10000 cfu/ml. This shows that the determination of differential bacterial counts can help to indicate failures already before changes of TBC occur.
On farms L, M and N bacterial counts resulting from milking equipment may be overestimated due to low filling status of the bulk tank at time of sampling.

3.3 Bacterial counts in fresh bedding

If contamination of fresh bedding is high, bacterial contamination of teats is not necessarily associated to management of the barn. Therefore bacterial counts in fresh bedding material were analysed to determine the potential contribution of unused bedding to the bacterial load of teat surfaces. The results are shown per farm in figures 4 and 5.

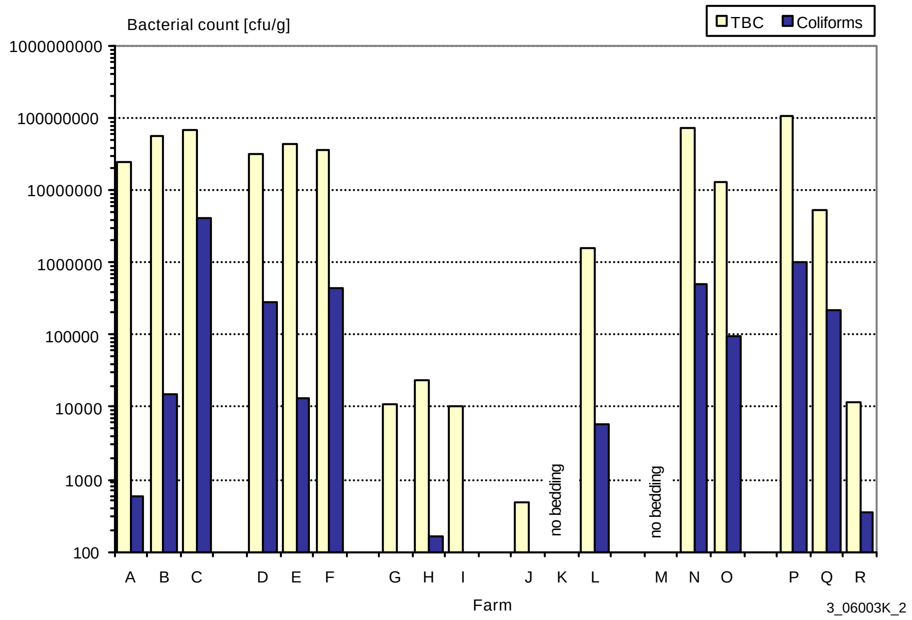

Figure 4: TBC and coliform bacteria in bedding material, per farm

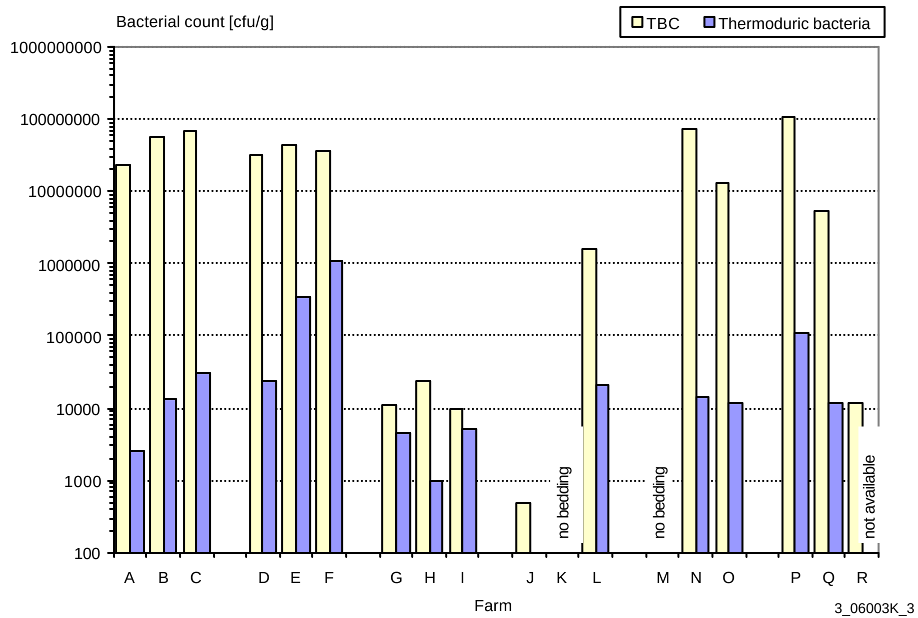

Figure 5: TBC and thermoduric bacteria in bedding material, per farm

Farms K and M used mattresses without any additional bedding. Differences in fresh bedding regarding content of bacteria were seen for TBC as well as for coliform and thermoduric bacteria.

The bacterial counts are ordered by type of bedding in figures 6 and 7. TBC in fresh bedding material varied between less than 10^3 cfu/g and 10^8 cfu/g. Lowest TBC were found in wood shavings of the brand Allspan® (special production and de-dusting process), in sand and in one additional sample of wood shavings. Large differences were observed regarding coliform counts even in the same kind of bedding material and with similar TBC (figure 6). Coliform bacteria were not detected in any of the 3 samples of Allspan® (detection limit 100 cfu/g).

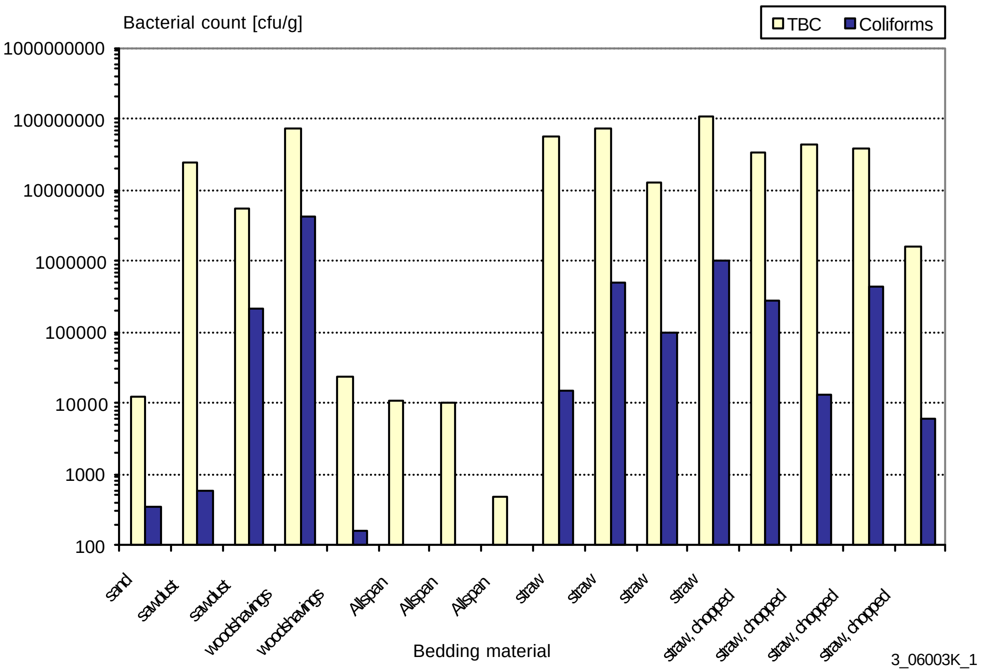

Figure 6: TBC and coliform bacteria in bedding material ordered by type of bedding

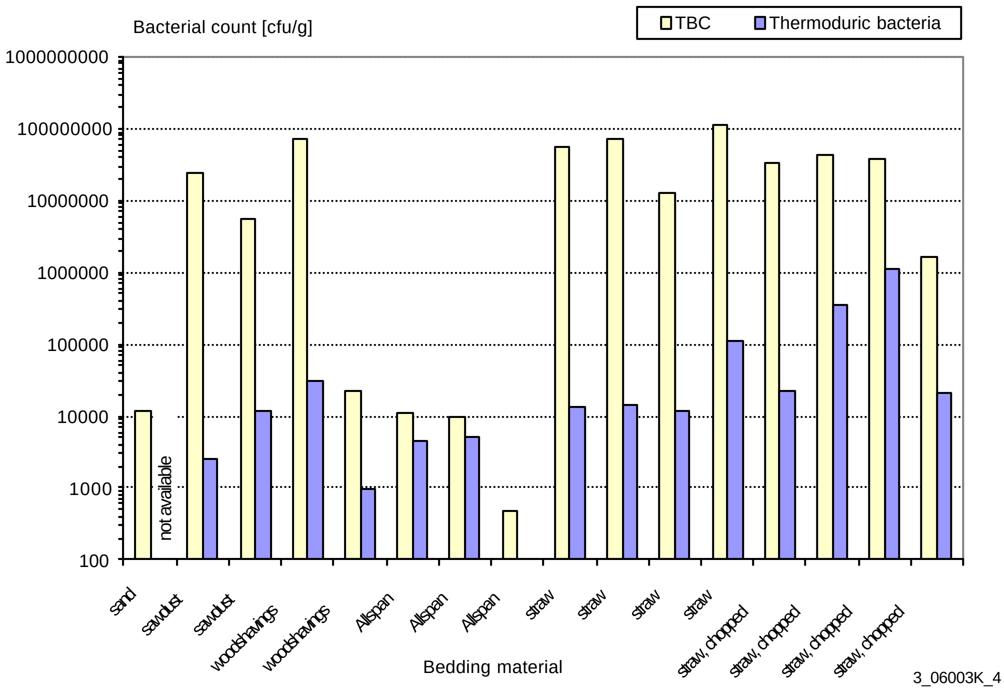

Figure 7: TBC and thermoduric bacteria in bedding material ordered by type of bedding

Thermoduric bacteria were detected in all samples of bedding materials except in one sample of Allspan®. These results show, that thermoduric bacteria in bulk milk may not only be due to insufficient system cleaning but can also originate from bedding material when udders are not sufficiently cleaned before milking.

Differences in coliform counts seemed to be more pronounced than differences in content of thermoduric bacteria. These differences may be due to conditions during harvesting of straw or during production of sawdust/wood shavings. The production process of Allspan® seems to reduce bacterial counts very effectively.
High bacterial counts in bedding may also develop due to unsuitable storing conditions of bedding. For example on one farm the bedding was stored outside without shelter resulting in TBC of $2.4x10^7$ cfu/g.

Hogan et al. (1989) reported bacterial counts in used bedding material of up to 10^7-10^9 cfu/g. Our results show that bacterial load in this range can already be found in unused bedding. Coliform bacteria in bedding material are not necessarily caused by faecal contamination, because coliform bacteria were already detectable in unused bedding.

3.4 Questionnaire on farm management

In addition to brand of AM system differences between individual farms were found to be important for teat cleaning efficiency. Also the initial contamination of teats has a significant influence on teat cleaning efficiency, independent of AM brand. Therefore it is necessary to look at management practices on individual farms.
Some of the results of the questionnaire on general aspects of farm management are summarized in table 1.

Table 1: General aspects of management on farms with AM systems, results of questionnaire

Observation	No. of farms	Average	Min	Max
No. of cows in lactation	18	74	34	158
No of lactating cows per milking box				
single box systems	9	52	34	69
multi-box systems	9	35	26	43
Average milking frequency per cow per day	18	2.6	2.1	3.0
Time until cows are fetched (hours)	15	12.8	10	16
Ratio cubicles/cows	18	1.2	0.8	1.7
Ratio feeding places/cows	18	1.0	0.5	1.5
Feeding of roughage per day	18	1.5	1	3

Information on selected aspects potentially related to teat cleanliness is summarized in table 2. Two farms cleaned cubicles once per day, 13 farms twice and one farm three times per day. The frequency of adding new bedding was between once per month and twice per day. The ground in the lying area was slatted floor on 13 farms and concrete floor on 5 farms. In addition to the 5 farms with concrete floor manure scrapers were used on 4 farms with slatted floor. The frequency of scraping was between 4 and 12 times per day. Manual cleaning of floors was performed on 4 farms with scraper and on 4 farms without scraper. 4 farms had cows lying on alleys in the herd. The number of these cows was between one and 8 cows per herd representing 1 to 15 % of lactating cows.

Table 2: Special aspects of farm management with regard to teat cleanliness, results of questionnaire

Variable		No. of farms	yes	no
Disinfection of teat cleaning brushes		9	8	1
Use of teat spray after milking		18	12	6
Separation box for AM system in use		18	7	11
Disinfection of cubicles		18	9	9
Presence of cows lying on alleys		18	4	14
Use of cow brushes in the barn		18	15	3
Manual cleaning of udders		18	0	18
Shearing of udders		18	16	2
Shearing of tails		17	12	5
Cutting of tail brush		18	18	0
Selection of cows for	udder shape	18	9	9
	robot acceptance	18	7	11
	activity	18	7	11
	udder health	18	15	3
	claw health	18	11	7

The frequency of replacing the teat cleaning device ranged from every 6 weeks to every second year. None of the farmers cleaned the udders manually. The frequency of claw trimming varied between once per year (7 farms), twice per year (5 farms) and 3 times per year (3 farms), no information was given on 3 farms.

3.5 Checklist on hygiene status

The results of the hygiene checklist are presented in table 3.

Table 3: Results of hygiene checklist (18 farms)

Aspect	No of farms	No. of farms with score		
	total	1	2	3
First impression of farm	18	12	5	1
First impression of barn	18	14	3	1
First impression of robot	18	10	7	1
Floor of milking boxes	18	13	5	0
Teat cups	18	9	7	2
Robot arm	18	10	7	1
Teat cleaning device	17	9	6	2
Waiting area for cows	18	6	8	4
Walking area beneath robot	8	5	2	1
Feeding area	18	17	1	0
Floor of barn	18	10	8	0
Water troughs	18	11	6	1
Cubicles	17	14	2	1
Quality of bedding	16	13	1	2
General cleanliness of cows	17	14	2	1
Shearing of udders	17	12	3	2
Condition of claws	16	8	7	1

Single use paper was available next to the robot on 8 of 18 farms.
It seems that the aspects waiting area, teat cups, teat cleaning device and condition of claws were of minor importance for farmers. These aspects were scored moderate or poor on approximately 50 % of farms.
Cleanliness of the feeding area was excluded from further analysis because it was scored as good on 17 of 18 farms.
Some of the results may be biased due to extra cleaning efforts of the farmers before the visit, although this did not seem to be common. However, one farmer reported to have cleaned the AM system the day before the visit.

3.6 Analysis of variance

The results of the analysis of variance regarding influence of different factors on teat contamination are shown in table 4. Factors are described in detail in Annex II. In table 4 only those factors are presented which were significantly associated with teat contamination before cleaning, either by evaluation based on determination of TBC or ATP.

Table 4: Management factors significantly associated with level of teat contamination

Management factor	% of variance explained[1] TBC	ATP	Higher teat contamination if
Parameters of questionnaire			
Replacement of teat cleaning device	24.2*	20.5*	$\leq$ 1x per year
Average milking frequency	19.7*	10.7	$\leq$ 2.5/day
Selection of cows for robot acceptance	19.2*	12.1	no
Ratio cubicles/cows	21.7*	16.0*	<1
Presence of cows lying on floor	16.1*	18.4*	yes
Addition of fresh bedding material	22.7*	10.1	< 1x per day
Selection of cows for udder health	27.8*	13.7	no
Use of cow brushes in the barn	28.9*	16.9*	no
Cleaning of milking box	5.2	24.5*	< 2x/day
Parameters of hygiene checklist			
Score - teat cleaning device	4.4	17.1*	$\geq$ 2
Score – quality of bedding	35.4*	19.9*	$\geq$ 2
Score – claw condition	46.7*	3.0	$\geq$ 2
Score - impression of robot	8.9	30.2*	$\geq$ 2
Score - teat cups	7.4	18.7*	$\geq$ 2

[1] Calculations based on mean teat contamination per farm before cleaning, either based on TBC [Log_{10} cfu/ml] or ATP [Log_{10} RLU]; * significant association with teat cleanliness (p<0.10)

Cubicle dimensions were excluded from the analysis because recommendations vary very much between different authors and for different body weights of cows (Anderson, 2003). Between farms included in the study there was also variation in breeds (Holstein Black and White, Red Holstein). Due to these reasons it was difficult to set a common standard. It can be expected that cubicle dimensions have an important influence on cow cleanliness. On the other side Sunderland et al. (2002) found acceptable cleanliness for more than 50 % of cows although 100 % of cubicles failed to achieve a satisfactory total cubicle score when dimensions were evaluated in relation to cow cleanliness. No single factor of cubicle dimensions could be determined with overall significance for cow cleanliness in that study.

Some of the factors presented in table 4 were significantly associated with teat contamination only when the evaluations was based on either TBC or ATP. This is probably due to the fact that some factors are more directly associated with bacterial contamination of teats whereas others affect other kinds of teat contamination than bacteria.

Some of the important management factors are directly related to the teat cleaning process itself. Maintenance of teat cleaning devices in not too long intervals is important because devices loose their function with age and may lead to increased teat contamination levels over time. Checking the visual appearance of teat cleaning devices may give indications for reduced cleaning efficiency. In addition, high levels of teat contamination can be responsible for a poor hygiene status of the teat cleaning device if the device is not cleaned in regular intervals.

A high average milking frequency is associated with more frequent cleaning of teats and as a consequence reduced levels of teat contamination. For the same reason selection of cows for robot acceptance may be important, because it can be assumed that cows not visiting the AM system voluntarily have a lower frequency of milking and teat cleaning. Unfortunately no data were available on average milking frequency of individual cows sampled during the investigation.

A number of factors is related to cleanliness of cubicles. Teats are likely to be less contaminated if clean cubicles are available. If less than one cubicle per cow is available the number of occupied stalls is probably high. Gaworski et al. (2003) determined a positive correlation between stall usage and stall contamination. Opportunities for cows to select clean cubicles are reduced if the number of free cubicles is low. More intensive efforts to clean cubicles may be necessary to compensate for higher stocking densities.
If severe restrictions for lying down or getting up are present cows may prefer not to use the cubicles, but to lie on the alleys which leads to higher teat contamination. Therefore the presence of cows lying on alleys can be interpreted as an obvious sign for improper cubicle design. Other reasons may be improper training of heifers to use cubicles.
An extreme situation was observed on one farm where cows were kept in the former barn for heifers. Cubicles were far too small and cows had serious problems in lying down or getting up. On this farm 15 % of cows preferred lying on the alleys.

Total bacterial counts in fresh bedding had no significant influence on contamination of teats, but the quality of bedding material as determined by visual evaluation was important. When visual appearance of bedding was scored as moderate or poor it was probably of very bad quality thus contributing to higher teat contamination.
More important than TBC in unused bedding is probably the bacterial load in used bedding material which is influenced by the frequency of adding new bedding. Addition of fresh bedding material to cubicles at least once per day reduced bacterial contamination of teats. Fresh bedding limits facilities for bacterial growth due to less humidity.
A correlation between contamination of lying area surface and contamination of teat surfaces was observed by Reithmeier et al. (2004), but only for enterococci and anaerobic spore formers. Also the general cow cleanliness may be influenced by the quality of used bedding. Sunderland et al. (2002) found a significant association between soiled bedding and higher flank contamination.
Cows with mastitis may contaminate cubicles with high numbers of bacteria when leaking of milk occurs. Selection of cows for udder health may reduce the contamination risk of cubicles and is indirectly associated with lower bacterial counts on teats. Selection of cows for udder health could also be part of a mastitis control programme in addition to providing a clean environment, which is more directly associated with teat contamination. Good housing conditions will also reduce the risk for mastitis. Reneau et al. (2003) determined a correlation be-

tween hygiene scores of udder and rear legs/feet with somatic cell count in milk of individual cows.

The association between use of cow brushes in the barn and teat cleanliness is difficult to explain. When no brushes are used this can be interpreted as a sign that cow comfort plays a minor role for the farmer. Also the cleaning of the milking box has probably no direct influence on teat cleanliness, but more likely reflects the general attitude of the farmer towards hygiene as does the general impression of the robot. The hygiene status of teat cups seems to be a consequence of teat contamination rather than a cause. However, the outer surfaces of teat cups can contribute to teat contamination during attachment of the milking cluster as well.

Remarkable is that claw condition explained 47 % of variance when the evaluation was based on bacterial contamination of teats. One explanation for these findings is that when claw condition is poor, cows may contaminate their udders more easily e.g. by more frequent lying. Difficulties during lying down or getting up could also be a cause for more contacts between claws and udder in such cows. In addition, poor claw condition may negatively influence milking frequency and therefore cleaning frequency of teats. More likely bad claw conditions are an expression of very unsuitable conditions in the barn which negatively affect claw health and teat contamination. Significant associations between udder and leg hygiene scores were determined by Schreiner and Ruegg (2003). It can be assumed that with good management of animal health also more attention is paid on cow comfort and cow cleanliness.

In interpreting the results it should not be forgotten that for some management factors which are expected to be important for teat cleanliness no variability occurred between farms and were therefore not included into the analysis of variance. The fact that the majority of farms practised cubicle cleaning twice per day, shearing of udders and cutting of tail brushes shows that these are generally accepted hygiene measures to keep cows clean.

In this study no efforts were made to determine the correlation between teat cleanliness or teat cleaning efficiency and milk quality, because only one bulk milk sample was available for determination of differential bacterial counts. Also data on total bacterial counts were not available on all farms throughout a longer period and TBC gives only restricted information on potential causes of failures in milk quality. Results from earlier investigations showed that even with a high number of farms it is difficult to show any relationship of management factors to milk quality. Reithmeier et al. (2004) could only prove a relationship between contamination of teats, bedding material and milk quality for anaerobic spore formers but not for other bacteria. During an investigation including 124 farms Van der Vorst and Ouweltjes (2003) detected no influence of management on milk quality. This is probably due to the multiple factors influencing milk quality making the determination of the contribution of single factors difficult or impossible.

Carry over of material from teat surfaces into milk can be reduced by milking techniques which effectively restrict teat washing during milking. Other origins than mastitis pathogens and bacteria from milk contact surfaces contribute significantly to bacterial counts of bulk tank milk, especially if a mastitis problem or failures in system cleaning exist. In addition, effective cooling can limit bacterial growth during milk storage.

The fact, that in this study an association between management factors and teat contamination was determined is probably due to the fact that these are more closely related.

In general, farmers may be able to compensate for sub-optimal conditions by appropriate hygiene management, whereas the best conditions will not maintain cows clean if maintenance is neglected. Appropriate construction of barns can support farmers but recommendations are not yet uniform and a lot of research is currently performed to improve housing conditions.

4 Conclusions

The evaluation of teat cleaning efficiency on different farms working with AM systems of 6 different brands revealed that although differences between the different systems exist management factors are equally important to ensure clean teats before milking. Cows entering the AM system with very dirty teats will not be cleaned sufficiently and may contribute to high bacterial counts in bulk tank milk.

High coliform counts in bulk milk of individual farms indicated insufficient teat cleaning and/or failures in system cleaning on 8 farms. By determination of differential bacterial counts problems with milk quality may be detected even before TBC in bulk milk increases above 10000 cfu/ml. Differential bacterial counts also give hints for potential origins of a problem.

The evaluation of management practices showed a significant association of certain factors with the average teat contamination level on farms.
AM specific management factors associated with high teat contamination were: replacement of teat cleaning device $\leq$ once per year, moderate/poor status of the teat cleaning device, average milking frequency per day $\leq$ 2.5 and no selection of cows for robot acceptance ($p<0.10$). Factors not directly related to AM involved contamination of cubicles: less than one cubicle per cow, cows lying on alleys present in the herd, addition of fresh bedding material < once per day, no selection of cows for udder health, moderate/poor status of bedding material and moderate/poor status of claws were significantly related to high teat contamination.

Additional factors like the general impression of the robot, cleaning frequency of the milking box, status of teat cups and the use of cow brushes in the barn were probably more closely related to the general attitude of the farmer towards hygiene than to teat cleanliness.
Other factors can be regarded as generally accepted hygiene measures, because nearly all farmers practised cubicle cleaning twice per day, shearing of udders and cutting of tail heads. These measures should therefore be a basis to which other management factors are added.

In interpreting these data it has to be regarded that only 18 farms could be included into the study. The investigation of management factors was based on farmers´ answers to a questionnaire without any means to check for reliability of these data. In several cases values had to be combined to one value to approximate a more uniform distribution.

Although the cause and effect relationship between parameters used to evaluate hygiene management on farms and teat cleanliness was not always very strong these factors should be considered when improvements of teat cleanliness are intended. Even with very good conditions it will be unavoidable that individual cows will have soiled teats, but farm management should aim for clean udders in the majority of cows. Apart from those factors which are specific for AM systems (e.g. frequency of replacing of teat cleaning devices, milking frequency etc.) it can be expected that the other factors can also improve teat cleanliness on farms with conventional milking systems.

5 Bibliography

Anderson, N.G. (2003) *Observations on dairy cow comfort: diagonal lunging, resting, standing and perching in free stalls*. Proceedings of the Fifth Dairy Housing Conference, 29-31 January 2003, Fort Worth, Texas, pp. 26-35

Council Directive 89/362/EEC of 26 May 1989 on general conditions of hygiene in milk production holdings, Official Journal of the European Communities No. L156, 08.06.89, p. 30-32

Council Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat treated milk and milk-based products, Official Journal of the European Communities No. L268, 14.09.1992, pp. 1-32

Frank, J.F., Hankin, L., Koburger, J.A., Marth, E.H. (1985) *Tests for groups of microorganisms*. In: Richardson, G. H. (Hrsg.) Standard methods for the examination of dairy products, 15th Edition, American Public Health Association, Washington, D. C., 189-191

Gaworski, M.A., Tucker, C.B., Weary, D.M., Swift, M.L. (2003) *Effects of stall design on dairy cattle behaviour*. Proceedings of the Fifth Dairy Housing Conference, 29-31 January 2003, Fort Worth, Texas, pp. 139-146

Hansen, S.R. (1973) *Studies on the influence of udder cleaning methods on the bacterial flora of the teat skin, the mastitis incidence and the bacterial contamination of the bulk milk*. Nord. Vet.-Med. 25, 359-371

Hogan, J.S., Smith, K.L., Hoblet, K.H., Todhunter, D.A., Schoenberger, P.S., Hueston, W.D., Pritchard, D.E., Bowman, G.L., Heider, L.E., Brockett, B.I., Conrad, H.R. (1989) *Bacterial counts in bedding materials used in nine commercial dairies*. J. Dairy Sci. 72, 250-258

International Dairy Federation (1985) *Milk and milk products, Enumeration of coliforms - Colony count technique and most probable number technique at 30 °C*. IDF Standard 73A:1985

International Dairy Federation (1991) *Milk and milk products, Enumeration of microorganisms - Colony count technique at 30 °C*. IDF Standard 100B:1991

Knappstein, K., Roth, N., Slaghuis, B.A., Ferwerda-van Zonnefeld, R.T. (2002) *Effectiveness of automatic cleaning of udder and teats and effects of hygiene management. Protocol for evaluation of teat cleaning systems*. Report D13, EU-Project Implications of the introduction of automatic milking on dairy farms. (QLK5-2000-31006), http://www.automaticmilking.nl

Knappstein, K., Roth, N., Walte, H.-G., Reichmuth, J., Slaghuis, BA., Ferwerda-van Zonneveld, R.T., Mooiweer, A. (2003) *Effectiveness of automatic cleaning of udder and teats and effects of hygiene management. Report on effectiveness of cleaning procedures applied in different automatic milking systems*. Report D14, EU-Project Implications of the introduction of automatic milking on dairy farms (QLK5-2000-31006), http://www.automaticmilking.nl

Reinemann, D. (1997) *Troubleshooting high bacteria counts in farm milk*. Proc. 36th Annual Meeting, National Mastitis Council, Inc., Albuquerque, New Mexico, pp. 65-79

Reithmeier, P., Schaeeren, W., Schällibaum, M., Friedli, K. (2004) *Bacterial load of several lying area surfaces in cubicle housing systems on dairy farms and its influence on milk quality*. Milchwiss. 59, 20-24

Reneau, J.K., Seykora, A.J., Heins, B.J., Bey, R.F., Farnsworth, R.J. (2003) *Relationship of cow hygiene scores and SCC*. Proc. 42th Annual Meeting, National Mastitis Council, Inc., Fort Worth, Texas, pp. 362-363

Schreiner, D.A., Ruegg, P.L. (2003) *Relationship between udder and leg hygiene scores and subclinical mastitis*. J. Dairy Sci. 86: 3460-3465

Slaghuis, B. (1996) *Sources and significance of contaminants on different levels of raw milk production*. Proc. IDF Symp. Bacteriological quality of raw milk, Wolfpassing, Austria, p. 19-27

Sumner, J., 1996. *Farm production influences on milk hygiene quality*. Proceedings of the IDF Symposium on Bacteriological Quality of Raw Milk. Wolfpassing, Austria, pp. 94-102.

Sunderland, E., Browne, R., McKinney (2002) *A study of the association between cattle cubicle design and cow cleanliness*. BCVA 10, 147-155

Van der Vorst, Y., Bos, K., Ouweltjes, W., Poelarends, J. (2003) *Milk quality on farms with an automatic milking system- Farm and management factors affecting milk quality*. Report D9, EU project Implications of the introduction of automatic milking on dairy farms. (QLK5-2000-31006), http://www.automaticmilking.nl

Van der Vorst, Y., Ouweltjes, W. (2003) *Milk quality and Automatic Milking: a risk inventory*. Report 28, Praktijkonderzoek Veehouderij, Lelystad, The Netherlands, ISSN 1570-8616

6 Annex I

In the following the hygiene scores are explained which were used in the checklist on different areas of farms with AM systems. The evaluation was based on the scheme developed by Van der Vorst et al. (2003) for hygiene checks on farms with some modifications.

First impression of farm	
Good:	Tidy, clean, well swept
Moderate:	Somewhat untidy, not well swept
Poor:	Chaotic, not swept for some time, remains of feed on road
First impression of barn	
Good:	Clean feeding passage, dry, no remains of fodder, tidy
Moderate:	Dung, remains of fodder, somewhat untidy
Poor:	Dung, remains of fodder, damp, dirty, untidy
First impression of robot	
Good:	Clean floor, walls, hoses and equipment
Moderate:	Dung on floor, dried dung on walls and hoses, lightly soiled equipment
Poor:	Much dung (old/fresh) on the floor and walls, dung and milk residues on equipment, cobwebs, thick dust-layer
Floor of milking boxes	
Good:	Fresh dung only
Moderate:	Much dung, dried dung in corners
Poor:	Much dung (fresh/old), fodder and bedding
Teat cups	
Good:	Clean
Moderate:	Splashed with fresh dirt
Poor:	Dirty teat cups, much dried dung, much milk
Robot arm	
Good:	Clean
Moderate:	Splashed with fresh dirt and/or damaged
Poor:	Dirty, much dried dung and milk and/or damaged
Teat cleaning device	
Good:	Clean
Moderate:	Traces of dung
Poor:	Traces of dung and/or milk, worn brushes
Waiting area for cows	
Good:	Fresh dung only
Moderate:	Much dung, dried dung in corners
Poor:	Much dung (fresh/old), fodder and bedding
Walking area beneath robot	
Good:	Fresh dung only
Moderate:	Much dung, dried dung in corners
Poor:	Much dung (fresh/old), fodder and bedding

Feeding area
Good: Clean and dry, structurally sound
Moderate: Old feed residues, muddy here and there, structurally sound
Poor: Old feed residues, muddy, dung, damp, smelly, structurally damaged

Floor of barn
Good: Dung-free
Moderate: Lots of dung, dried dung in corners
Poor: Lots of dung (fresh/old), fodder and bedding, puddles of liquid manure

Water troughs
Good: Clean water, no sediment on bottom
Moderate: Turbid water, sediment on bottom (up to 5 cm), water smells stale
Poor: Turbid water, bottom not visible, more than 5 cm sediment on bottom, water and sediment smelly

Cubicles
Good: Majority of cubicles clean and dry, fresh bedding
Moderate: Majority of cubicles with some dung, urine or milk
Poor: Majority of cubicles smeared with much dung, urine, milk and old bedding

Quality of bedding
Good: Storage dry, under roof, clean, good smell
Moderate: Storage under roof or covered, damp, musty smell
Poor: Storage not under roof or covered, damp, dirty, musty smell

General cleanliness of cows
Good: Majority of cows with clean and shaved hindquarters and tail
Moderate: Majority of cows smeared with dung and urine
Poor: Majority of cows smeared with much wet dung and urine

Shearing of udders
Good: Udder just sheared
Moderate: Udder sheared some time ago
Poor: Udder not sheared

Claw condition
Good: Majority of cows with claws in good care condition
Moderate: Majority of cows with claws in moderate care condition
Poor: Majority of cows with claws in poor care condition

7 Annex II

In the following the variables used for the analysis of variance are listed together with the number of farms for which information was available for the respective parameter and the codes used to summarize the results.

General housing	n	Coding
Ratio cows/cubicles	18	<1, ≥1
Ratio cows/feeding places	18	<1, ≥1
Separation between feeding and lying area	17	yes, no
Separation box for AM system in use	18	yes, no
Number of calving boxes	18	≤1, >1
Cubicles in calf rearing area	18	yes, no

AM system	n	Coding
Average milking frequency per day	18	≤2.5, > 2.5
Minimum milking interval (hours)	15	≤ 5, > 5
Maximum time until cows are fetched (hours)	15	≤ 12, > 12
Conventional milking system available	18	yes, no
Duration of udder cleaning (seconds)	17	< 40, ≥ 40
Replacement of teat cleaning device per year	15	< 2 , ≥ 2
Use of teat dip	18	yes, no
Cleaning milking box per day	18	1, >1
Cleaning of robot arm per day	18	≤1, >1
Cleaning of waiting area per day	15	< 1x per month, ≥ 1x per month
Cleaning of walking area next to robot per day	18	≤1, >1

Lying area	n	Coding
Type of cubicles	18	mattress, deep bedded stalls, both
Floor of cubicles	18	concrete, mattress
Bedding	18	no bedding/sand, straw/chopped straw, saw-dust/wood shaving
Bacterial counts in bedding material [Log_{10}/g]	16	<7.0, ≥7.0
Addition of new bedding to cubicles per day	15	<1x, ≥1x
Desinfection of cubicles	18	yes, no
Floor of alleys in lying area	18	slatted floor, concrete floor
Manure scraper	18	yes, no
Frequency of cleaning of alleys per day (manure scraper and/or manual cleaning)	18	0, ≥1
Slatted floor plus manure scraper	13	yes, no

Feeding area	n	Coding
Floor feeding area	18	slatted floor, concrete floor
Manure scraper in feeding area	18	yes, no
Manure scraper and/or manual cleaning in feeding area	18	yes, no
Frequency of roughage supply per day	18	1, >1
Additional concentrate supply in the barn	18	yes, no

Cow management	n	Coding
Use of cow brushes in the barn	18	yes, no
Frequency of udder shearing per year	16	≤1, >1
Shearing of tails	17	yes, no
Frequency of tail shearing per year	15	≤1, >1
Frequency of cutting tail brush per year	18	≤1, >1
Regular interval for claw caring	18	yes, no
Frequency of claw caring per year	15	1, >1
Cows lying on alleys present in herd	18	yes, no
Selection for udder health	18	yes, no
Selection for robot acceptability	18	yes, no
Selection for udder health	18	yes, no
Selection for claw health	18	yes, no
Selection for activity	18	yes, no

Variables of checklist	n	Coding
First impression of farm	18	1, >1
First impression of barn	18	1, >1
First impression of robot	18	1, >1
Floor of barn	18	1, >1
Cubicles	17	1, >1
Quality of bedding	16	1, >1
Water troughs	18	1, >1
Waiting area for cows	18	1, >1
Walking area beneath robot	8	1, >1
Floor of milking boxes	18	1, >1
Teat cups	18	1, >1
Robot arm	18	1, >1
Teat cleaning device	17	1, >1
General cleanliness of cows	17	1, >1
Shearing of udders	17	1, >1
Condition of claws	16	1, >1
Single use paper available at robot	18	yes, no

Acknowledgements

The authors wish to thank the representatives of the AM brands for their support by providing the addresses of farmers and establishing contacts. The farmers are gratefully acknowledged for their cooperation in the on-farm studies. In addition, we want to express our thanks to the laboratory technicians involved, especially to Sandra Soll-Gnutzmann for excellent technical assistance and to Burkhard Nipp for assistance in data filing.

Abbreviations

AM	Automatic Milking
ATP	Adenosine-tri-phosphate
cfu	Colony forming units
GLM	General Linear Model
IDF	International Dairy Federation
RLU	Relative Light Units
TBC	Total bacterial count