



## Relationship between udder health and hygiene on farms with an automatic milking system

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### ABSTRACT

Poor hygiene is an important risk factor for reduced udder health. Because the teat cleaning process is done automatically on farms with an automatic milking system (AMS), hygiene management might differ. The aim of this study was to determine the relationship between hygiene and udder health on farms with an AMS at the farm level as well as at the cow level. Information on hygiene and udder health was collected on 151 Dutch dairy farms with an AMS. Teams of 2 veterinary students collected data with the use of a partially open-ended questionnaire and scoring protocols for hygiene of the cows, cleanliness of the AMS, and functioning of the AMS. Milk production records from the Dutch dairy herd information association were also collected. Stepwise general linear models were used to analyze the relation between hygiene and udder health at farm level. Dependent variables were average herd somatic cell count (SCC), the average percentage of new cows with a high SCC, and the incidence rate of clinical mastitis, all in the year preceding the farm visit. The annual average herd SCC was positively related to the proportion of cows with dirty teats before milking and the proportion of cows with dirty thighs. The annual average percentage of new cows with a high SCC was positively related to the proportion of cows with dirty teats before milking and the proportion of milkings where teats were not covered with teat disinfecting spray by the AMS. The annual incidence rate of clinical mastitis was positively related to the frequency of replacing the milking filters. At the cow level, hygiene scores of the udder, thighs, and legs (range 1 to 4, where 1 is clean and 4 is very dirty) were related with cow SCC from the milk production test day closest to the farm visit using a general linear mixed model. The relationship between cow SCC and the hygiene score of the udder was positive.

**Key words:** udder health, hygiene, automatic milking system

### INTRODUCTION

The first automatic milking system (AMS) on a commercial farm was introduced in the Netherlands in 1992 (De Koning and Rodenburg, 2004). At the end of 2009, more than 8,000 commercial farms worldwide were milking with an AMS. In the Netherlands, almost 2,000 farmers are milking automatically (De Koning, 2010). Mastitis is a frequent and costly problem in many dairy herds (e.g., Halasa et al., 2007). In a recent study on conventional farms in the Netherlands, the average incidence rate of clinical mastitis (CM) was 30.3 cases/100 cows at risk per farm per year and the average bulk milk SCC (BMSCC) was 192,000 cells/mL (Jansen et al., 2009).

Udder health is at risk on farms with an AMS. Several studies have been published regarding the increase in BMSCC after the transition from conventional milking to automatic milking (AM; e.g., Van der Vorst and Hogeveen, 2000; Rasmussen et al., 2002). However, De Koning et al. (2004) found that BMSCC is increased only during the first 6 mo after transition. According to Klungel et al. (2000), BMSCC did not increase after introducing AM but was already higher before the change of system compared with other conventional herds. Contrary to BMSCC, quarter SCC decreased in an experimental study where AM was compared with conventional milking (Berglund et al., 2002). Any conclusions about the factors that cause these results and explain the differences found are hard to draw. Moreover, many more aspects than just milking technique change in the transition of the herd from conventional milking to AM (Poelarends et al., 2004).

On farms with a conventional milking system, BMSCC was lower when more attention was paid to hygiene management (Barkema et al., 1999). Schreiner and Ruegg (2003) found that udder hygiene scores and leg hygiene scores were significantly associated with cow SCC on 1,250 lactating dairy cows from 8 farms. An-

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other observational study on 1,093 lactating dairy cows from 8 farms showed significant relationships between cow SCC and hygiene scores of the udder and lower legs and the udder-lower leg composite score (Reneau et al., 2005). According to these results, hygiene aspects are also expected to be of importance in relation to SCC on farms with an AMS. Hygiene and hygiene management might even be more important because the automatic cleaning of the udder is a standardized process, so the cleaning of the udder cannot be adjusted to the dirtiness of individual cows. Some research had been done on the influence of poor hygiene on udder health on farms with an AMS, but knowledge on this subject is still poor. An observational study on 28 farms with an AMS in the Netherlands, designed to identify risk factors affecting milk quality, showed an increased BMSCC on farms with a poor overall hygiene (De Koning et al., 2003). Knapstein et al. (2004) determined significant differences in teat cleaning efficiency of different brands of AMS by measuring total bacterial count. Also, the initial contamination of teats had a significant influence on teat cleaning efficiency, independent of AMS brand. Several management factors associated with high teat contamination were found. However, no relationship was made with SCC and only 18 farms were included. The aim of the present study was to identify the relationship between hygiene and udder health on farms with an AMS.

## MATERIALS AND METHODS

### Data Collection

The Dutch dairy cooperative FrieslandCampina (Amersfoort, the Netherlands) approached 400 farms with an AMS in the Netherlands with the request to participate in the survey. From these 400 farms, 161 farms were willing to participate. From these 161 farms, 10 farms were excluded because they did not meet all the selection criteria for inclusion. Selection criteria were milking with an AMS for more than 1 yr, participation in the Dutch dairy herd information association, and no additional conventional milking. Finally, 151 farms were visited between May 2008 and November 2008. Data was collected during a 3-h farm visit using a partially open-ended questionnaire and scoring protocols for hygiene of the cows, cleanliness of the AMS, and functioning of the AMS. These 4 tools for collection are described in the next section. Each farm was visited by a team of 2 students from the Faculty of Veterinary Medicine (Utrecht University, Utrecht, the Netherlands). The questionnaire was explained to the students by experts who helped develop the survey. The students were trained by other experts in scoring

the hygiene of the cows, the cleanliness of the AMS, and the functioning of the AMS with the use of the 3 scoring protocols.

The Dutch dairy herd information association (Coöperatie Rundvee Verbetering, Arnhem, the Netherlands) collects milk production information on farms every 4 or 6 wk. They provided the milk production data, including cow identification, date of milk recording, test-day milk yields, and SCC for all cows. For each farm the milk production records in the year preceding the farm visit and the available milk production records after the farm visit were selected.

### Survey Design

The questionnaire consisted of 45 questions divided in 5 parts: general information, AMS, housing, cow hygiene, and udder health. Definitions of variables (e.g., the definition of CM) as we used them were discussed while conducting the questionnaire. The contents of the 5 parts of the questionnaire are summarized in Table 1.

The 3 scoring protocols were used to gain information about the cleanliness of the AMS parts, the functioning of the AMS, and cow hygiene by visual inspection. The cleanliness of 8 AMS parts was scored for each robot present on the farm (range 1 to 4, where 1 = clean and 4 = very dirty). The functioning of the AMS was scored for 10 milkings at every farm. The functioning was measured by scoring the cleanliness of the teats before and after milking and by scoring 6 different procedures of the AMS, all with different scoring systems. Cow hygiene was scored for at least 10 lactating cows at every farm. Hygiene of the udder, thighs (upper portion of the hind limbs), and legs (lower portion of the hind limbs), was compared with model animals depicted in photographs on the hygiene scoring protocol and scored (range 1 to 4, where 1 = completely free of or has very little dirt, 2 = slightly dirty, 3 = mostly covered in dirt, and 4 = completely covered, caked-on dirt; Schreiner and Ruegg, 2003). The contents and scoring systems of the 3 scoring protocols are listed in Table 2.

### Data Preparation

From the total of 151 visited farms, data from 7 farms were excluded from further analysis. From these farms, 4 farms were excluded because they had been milking with an AMS for less than 1 yr, 1 farm was excluded because no milk production information was available, 1 farm was excluded because cows were also milked conventionally, and 1 farm was left out because of a too-high proportion of missing values and some unlikely values (e.g., 0% of CM cases in the past year).

**Table 1.** Contents of the 5 parts of the partially open-ended questionnaire, divided into themes and subdivided into topics of questions

Part	Theme and topic of questions
Farm characteristics	Number of dairy cows Milk production Way of business (conventional or organic)
Automatic milking system (AMS)	Brand of the AMS Cleaning AMS space Different parts of the AMS Automatic cleaning of the AMS Premilking teat cleaning system Disinfection Replacement of the roller brush system or lining of the cleaning cup Functioning teat cleaning process Treatment after milking Disinfection Functioning teat spraying process Milking filters Replacement
Housing	Cubicles Number Ground layer Bedding material Cleaning Alleys Flooring type Cleaning Calving area Facilities Cleaning
Cow hygiene	Shaving/flaming cows/udders/tails Cows leaking milk
Udder health	Clinical mastitis Number of cases

From the milk production data, records with an SCC of 0, or 0 kg of milk, or both, were deleted (5% of all records).

Some transformations were made to the farm level data set (data set 1). The scores on the cleanliness of the AMS parts were averaged by farm and classified into clean (score 1) or not clean (score >1). The scores on functioning of the AMS and cow hygiene were calculated into proportions of the observations above a specific score by farm. The choice of the score used as threshold to transform a categorical score variable into a proportion differed between variables and was based on the significance of the relationship with the dependent variables shown by univariate statistical analysis. Besides the hygiene-specific variables, the time interval (4 or 6 wk) between the milk production test days was also determined for each farm.

For analysis at the farm level, 4 dependent variables on udder health were determined. The first 3 dependent udder health variables were calculated based on the information from the Dutch dairy herd information association. Milk production test days in the year preceding the farm visit were selected. Milk production test days with fewer than 10 milkings were deleted. Annual average herd SCC was calculated for each farm

as follows. First, the arithmetic mean of the SCC of the cows was calculated per milk production test day. Subsequently, the annual average herd SCC was calculated by taking the arithmetic mean of the average milk production test day SCC for the year preceding the farm visit. High SCC (**HSCC**) was defined as an individual cell count >150,000 cells/mL for primiparous cows and >250,000 cells/mL for multiparous cows, according to the currently used cut-off levels for sub-clinical mastitis in the Netherlands. These cut-off levels are based on the shape of the lactation curve for SCC, which differ significantly among parities. Multiparous cows have, generally, a higher SCC on each DIM than primiparous cows (Scheepers et al., 1997; de Haas et al., 2002). The annual average percentage of cows with HSCC was calculated on each farm as follows. First, the percentage of cows with HSCC was determined per milk production test day by dividing the number of cows with a high SCC by the number of cows tested. Subsequently, the annual average percentage of cows with HSCC was calculated by taking the arithmetic mean of the milk production test-day percentages of cows with HSCC for the year preceding the farm visit. The annual average percentage of new cows with HSCC (**NHSCC**) was calculated on each farm as follows.

**Table 2.** Contents and scoring systems of the 3 scoring protocols

Scoring protocol	Part	Scoring system
Cleanliness of automatic milking system parts	Camera or laser, milking tubes, teat cups, air inlets, robot arm, feeding trough, floor, premilking teat cleaning system	1 = clean 2 = slightly dirty 3 = dirty 4 = very dirty
Functioning of automatic milking system	Cleanliness of the teats before milking	1 = clean 2 = slightly dirty 3 = dirty 4 = very dirty
	Frequency of teat cleaning process per milking	Times/milking
	Number of teats cleaned per milking	1-4
	Cleanliness of the teats after cleaning	1 = clean 2 = slightly dirty 3 = dirty 4 = very dirty
	Self-cleaning process of the roller brush system	1 = good 2 = tolerable 3 = moderate 4 = no cleaning
	Amount of surface of teats covered with disinfecting spray from the bottom up	1 = >60% 2 = 30-60% 3 = 0-30% 4 = no coverage
Cow hygiene	Self-cleaning process of the milking cluster	1 = good 2 = moderate 3 = no cleaning
	Steam cleaning of the milking cluster	1 = present 2 = absent
Cow hygiene	Udder, thighs, legs	1 = completely free of or has very little dirt 2 = slightly dirty 3 = mostly covered in dirt 4 = completely covered, caked-on dirt

First, the percentage NHSCC was determined per milk production test day by dividing the number of cows who had HSCC in the current milk production test day and low SCC in the previous milk production test day by the number of cows tested. Subsequently, the annual average percentage NHSCC was calculated by taking the arithmetic mean of the milk production test-day percentages NHSCC for the year preceding the farm visit. The fourth dependent udder health variable was based on the number of CM cases in the past year and the average number of cows present on the farm in the past year, both reported by the farmer. Annual incidence rate of CM was estimated as the number of CM cases per year divided by the average number of dairy cows present on the farm that year.

At cow level (data set 2), the data set contained milk production information for all cows within a range from 6 wk before until 6 wk after the farm visit. Dutch farmers can use different cow identification systems in parallel. Because of differences between barn identification of cows (used during data collection) and cow identification in the milk production data, for several farms the cow identification in the hygiene scoring data

could not be matched with milk production information. Farms where this happened were excluded from the data set. For the remaining cows, hygiene scores of the udder, thighs, and legs were available. Cows with double or unlikely hygiene scores were excluded. Besides the hygiene scores, the number of days between the farm visit and the milk production test day was determined for every cow in the final data set.

### Statistical Analyses

Before further statistical analysis at farm level, the associations between the hygiene-specific variables were investigated. The correlation between the continuous variables was studied using Pearson correlation. Relations between the categorical variables were analyzed using chi-squared analysis. High correlation ( $r > 0.95$ ) was seen between the 4 variables on the amount of surface of each teat covered with teat disinfecting spray from the bottom up as previously listed in Table 2. Therefore, they were averaged into 1 variable on the amount of surface of the 4 teats together covered with teat disinfecting spray. Furthermore, the proportion of

cows with a dirty udder, the proportion of cows with dirty thighs, and the proportion of cows with dirty legs were highly correlated ( $r = 0.78-0.88$ ). Because of our interest in the association between cow hygiene and udder health, none of the variables on cow hygiene were transformed or deleted. The correlation between the 4 udder health variables annual average herd SCC, annual average percentage cows with HSCC, annual average percentage NHSCC, and annual incidence rate of CM was studied using Pearson correlation. Annual average herd SCC and annual average percentage cows with HSCC were highly correlated ( $r = 0.89$ ), so the annual average percentage cows with HSCC was excluded for further analysis. The natural logarithm of the udder health variables annual average herd SCC, annual average percentage NHSCC, and annual incidence rate of CM was used for analysis. Finally, data set 1 consisted of 62 independent hygiene-specific variables and 3 dependent udder health variables measured on 144 farms. The variables in data set 1 and their unit, categories, or used threshold values are listed in Table A1.

At farm level, all hygiene-specific variables measured by the questionnaire and the scoring protocol were analyzed univariately for their relationship to the udder health variables annual average herd SCC, annual average percentage NHSCC, and annual incidence rate of CM using general linear models. Also at farm level, a selection of hygiene-specific variables from data set 1 was made based on their expected importance (biological relevance) and the amount of information known on that variable (maximum of 5 missing values). This last criterion was included so as not to lose too many farms because of missing values so the required power for the test could be retained. This selection was used to create 3 general linear models with the above-described udder health variables as outcome variables. For the udder health variable annual average percentage NHSCC, the time interval between the milk production test days was also included in the analysis. Correlation coefficients of the models were calculated. The udder health variables were determined by the following equation:

$$\text{Ln(UHV)} = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_i X_i + \text{group}_1 + \text{group}_2 + \dots + \text{group}_j + IV + \varepsilon,$$

where UHV = udder health variable (annual average herd SCC or annual average percentage NHSCC or annual incidence rate of CM),  $\alpha$  = intercept,  $\beta_i$  = regressive coefficient of continuous hygiene-specific variable  $X_i$ ,  $\text{group}_j$  = effect of categorical hygiene-specific variable, IV = time interval between milk production test days (included in the model only for annual average percentage NHSCC), and  $\varepsilon$  = residual random error.

Before further statistical analysis at cow level, the associations between the categorical variables were analyzed using chi-squared analysis. No variables needed to be transformed or excluded for this reason. One dependant variable on udder health for analysis at cow level was used. Cow SCC (CSCC) was determined for the cows involved in hygiene scoring by taking the individual CSCC from the nearest milk production test day within the previously described range of 6 wk before and 6 wk after the farm visit. The natural logarithm of CSCC was used for analysis. Finally, data set 2 consisted of 4 independent hygiene-specific variables and 1 dependent udder health variable measured on 2,294 cows from 108 farms. The variables in data set 2 and their unit, categories, or used cut-off values are listed in Table A2.

At cow level, the association between CSCC and hygiene scores of the udder, thighs, and legs was determined using a general linear mixed model. The number of days between the farm visit and the milk production test day was included in the analysis. Also, the interaction of the number of days with the hygiene scores was tested. The hygiene scores, the amount of days, and their products were fixed effects with herd as a random effect. To determine CSCC by hygiene scores the following equation was used:

$$\begin{aligned} \text{Ln (CSCC)} = & \alpha + \text{HU} + \text{HT} + \text{HL} + \text{DAYS} \\ & + (\text{HU} \times \text{DAYS}) + (\text{HT} \times \text{DAYS}) \\ & + (\text{HL} \times \text{DAYS}) + \mu_{\text{herd}} + \varepsilon, \end{aligned}$$

where  $\alpha$  = intercept; HU = hygiene score of the udder; HT = hygiene score of the thighs; HL = hygiene score of the legs; DAYS = number of days between farm visit and milk production test day;  $\text{HU} \times \text{DAYS}$ ,  $\text{HT} \times \text{DAYS}$ , and  $\text{HL} \times \text{DAYS}$  = interaction terms;  $\mu_{\text{herd}}$  = random herd effect; and  $\varepsilon$  = residual random error.

For both the farm level analysis and the cow level analysis, model selection was performed by using a backward stepwise procedure. The final models retained only the variables that were significant at  $P \leq 0.05$  using an  $F$ -test. Differences between groups in a significantly related categorical variable were tested for significance by pairwise comparison with the Student's  $t$ -test. Significance levels were multiplied by the number of comparisons made according to the Bonferroni correction (Abdi, 2007). The assumed normality was assessed by plotting the residuals in a normal probability plot, which were checked for peculiarities (Dohoo et al., 2003). All data preparation and statistical analyses were carried out using SAS (version 9.1, SAS Institute Inc., Cary, NC).

**Table 3.** General farm data and udder health variables

Variable	Farm level				Cow level			
	n	Mean	Minimum	Maximum	n	Mean	Minimum	Maximum
Dairy cows (n)	144	85	30	420	108	84	38	420
Milk production quota ( $\times 1,000$ kg)	142	796	154	5,000	106	798	165	5,000
305-d milk yield (kg)	144	9,008	5,500	11,000	108	9,041	6,200	11,000
Robot (n)	144	1.6	1	6	108	1.6	1	6
Cows/robot	144	54	30	85	108	55	33	80
Annual average herd SCC ( $\times 1,000$ cells/mL)	144	258 <sup>1</sup>	90	509	108	269	90	453
Annual average NHSCC <sup>2</sup> (%)	144	10	5	18	108	10	5	14
Annual incidence rate of clinical mastitis (cases/100 cows/yr)	144	26	1	135	106	28	1	135
Cow SCC ( $\times 1,000$ cells/mL)	NR <sup>3</sup>	NR	NR	NR	2,294	109 <sup>1</sup>	4	9,999

<sup>1</sup>Geometric mean.<sup>2</sup>NHSCC = new cows with a high SCC.<sup>3</sup>NR = not relevant.

## RESULTS

### Descriptive Statistics

Descriptive statistics of farm characteristics and the udder health variables of the farms and cows used in the analysis are listed in Table 3. For the 144 farms used in the analysis at farm level, the number of dairy cows on a farm ranged from 30 to 420, with 85 as an average. The annual milk production quota held between 154,000 and 5,000,000 kg, with an average of 796,000 kg. Average 305-d milk yield varied from 5,500 to 11,000 kg, with 9,008 kg as an average. There were 1 to 6 robots on a farm, with an average of 1.6. This means an average of 54 cows per robot, with a range from 30 to 85. The geometric mean of the annual average herd SCC was 258,000 cells/mL, with a range from 90,000 to 509,000 cells/mL. The annual average percentage NHSCC ranged from 5 to 18%, with an average of 10%. Annual incidence rate of CM varied from 1 to 135 cases/100 cows per year, with an average of 26 cases/100 cows per year. The 108 farms used for the cow level analysis were almost equal in farm characteristics to the 144 farms used for farm analysis. The geometric mean of CSCC for the 2,294 cows in cow analysis was 109,000 cells/mL, with a range from 4,000 up to 9,999,000 cells/mL (detection system does not measure levels above 9,999,000 cells/mL).

Of the 144 farms used in analysis at farm level, 4 farms were organic and the other 140 were conventional. Of the farms, 86 milked with an Astronaut (Lely Industries N.V., Maassluis, the Netherlands), 42 milked with a VMS (DeLaval International AB, Tumba, Sweden), and the other 16 farms milked with a Galaxy (SAC, Kolding, Denmark;  $n = 8$ ), an RMS (GEA Westfalia-Surge BV, Zeewolde, the Netherlands;  $n = 5$ ), or a Merlin (Fullwood Limited, Shropshire, UK;  $n = 3$ ). Two of the 108 farms used in analysis at cow level were organic farms, whereas the other 106 were conventional

farms. Of the farms, 67 milked with an Astronaut, 39 milked with a VMS, the remaining 2 farms milked with a Merlin.

Average hygiene scores of the different body parts and the distribution of the different scores are listed in Table 4. The average hygiene scores of the udder, thighs, and legs of the cows involved in analysis at cow level were 2.76, 2.54, and 2.45 respectively. Hygiene scores 2 and 3 were most frequently observed for the udder as well as for the thighs and legs.

### Univariate Analysis

The results of the univariate analysis on the relationship between the hygiene-specific variables and the udder health variables at farm level are listed in Table 5. Only hygiene-specific variables that were significantly related ( $P \leq 0.05$ ) to one or more udder health variables are listed. Annual average herd SCC was positively related to the cleaning frequency of the feeding trough, the proportion of cows with dirty teats before milking, the proportion of cows with a dirty udder, the proportion of cows with dirty thighs, and the proportion of cows with dirty legs. Annual average herd SCC was negatively related to the cleaning frequency of the laser or camera. Positive relationships were seen between annual average percentage NHSCC and the proportion of cows with dirty teats before milking, the proportion of milkings where teats were not covered with teat disinfecting spray, the proportion of cows with a dirty udder, and the proportion of cows with dirty thighs. Annual average percentage NHSCC was significantly higher when the milking tubes were not clean. Annual incidence rate of CM was positively related to the frequency of replacing the milking filters and the proportion of cows with dirty udders. The type of bedding material was also related to annual incidence rate of CM. When the

**Table 4.** Hygiene scores<sup>1</sup> of the udder, thighs, and legs of dairy cows

Item	Hygiene score		
	Udder	Thighs	Legs
Mean	2.76	2.54	2.45
Frequency of hygiene score <sup>2</sup>			
1	97 (4)	128 (6)	96 (4)
2	762 (33)	1,052 (46)	1,182 (51)
3	1,034 (45)	861 (37)	912 (40)
4	401 (18)	253 (11)	104 (5)

<sup>1</sup>1 = completely free of or has very little dirt; 2 = slightly dirty; 3 = mostly covered in dirt; 4 = completely covered, caked-on dirt.

<sup>2</sup>Values in parentheses are percentages.

bedding material was straw instead of sawdust, the annual incidence rate of CM was significantly higher. Annual incidence rate of CM turned out to be significantly higher when the feeding trough was determined to be not clean.

**General Linear Models**

The selection of 20 hygiene-specific variables from data set 1 used in multivariate analysis at farm level consisted of brand of the AMS, cleaning frequency of the teat cups and the teat cleaning system, frequency of replacing the milking filters, ratio cubicle:cow, bedding

material, refill of fresh bedding material, removal of wet spots and manure from the cubicles, nonautomated manure removal from the alleys, separate calving area, calving area used as hospital, shaving/flaming frequencies of the udder and tails, cleanliness of the teat cup, proportion of cows with dirty teats before milking, proportion of milkings where not all teats were cleaned, proportion of milkings where teats were not covered with teat disinfecting spray, proportion of cows with a dirty udder, proportion of cows with dirty thighs, and proportion of cows with dirty legs. More details on these variables and their unit or categories are given in Table A3.

**Table 5.** Results of the univariate analysis on the relationship between the hygiene-specific variables and the udder health variables at farm level<sup>1</sup>

Variable	Annual average herd SCC	Annual average percentage NHSCC <sup>2</sup>	Annual incidence rate of CM <sup>3</sup>
Cleaning frequency of the laser or camera	−*	NS	NS
Cleaning frequency of the feeding trough	+*	NS	NS
Frequency of replacing the milking filters	NS	NS	
≤1/d			−**
>1 ≤ 2/d			−*
3/d			Ref
Bedding material	NS	NS	
Straw			+*
Other			NS
None			NS
Sawdust			Ref
Cleanliness of the milking tubes	NS		NS
Clean		Ref	
Not clean		+*	
Cleanliness of the feeding trough	NS	NS	
Clean			Ref
Not clean			+*
Proportion of cows with dirty teats before milking	+**	+*	NS
Proportion of milkings teats not covered	NS	+**	NS
Proportion of cows with a dirty udder	+**	+**	+*
Proportion of cows with dirty thighs	+**	+*	NS
Proportion of cows with dirty legs	+**	NS	NS

<sup>1</sup>+ = positive significant relationship with dependent variable; − = negative significant relationship with dependent variable; Ref = reference category.

<sup>2</sup>NHSCC = new cows with a high SCC.

<sup>3</sup>CM = clinical mastitis.

\*P < 0.05; \*\*P < 0.01.

**Table 6.** Final general linear model for 3 udder health variables at farm level<sup>1</sup>

Variable	Annual average herd SCC			Annual average percentage NHSCC <sup>2</sup>			Annual incidence rate of CM <sup>3</sup>		
	$\beta$	SE	<i>P</i> -value	$\beta$	SE	<i>P</i> -value	$\beta$	SE	<i>P</i> -value
Intercept	12.051	0.137	<0.001	-2.429	0.034	<0.001	-1.073	0.213	<0.001
Frequency of replacing the milking filters	NR <sup>4</sup>	NR	NS	NR	NR	NS			
≤1/d							-0.747	0.229	0.003 <sup>5</sup>
>1 ≤ 2/d							-0.561	0.250	0.016
3/d							Ref <sup>6</sup>	—	—
Proportion of cows with dirty teats before milking	0.261	0.114	0.023	0.210	0.092	0.024	NR	NR	NS
Proportion of milkings teats not covered	NR	NR	NS	0.183	0.059	0.002	NR	NR	NS
Proportion of cows with dirty thighs	0.354	0.153	0.022	NR	NR	NS	NR	NR	NS

<sup>1</sup> $\beta$  = estimated regression coefficient.

<sup>2</sup>NHSCC = new cows with a high SCC.

<sup>3</sup>CM = clinical mastitis.

<sup>4</sup>NR = not relevant.

<sup>5</sup>Still significant after Bonferroni correction.

<sup>6</sup>Ref = reference category.

At farm level, the results of the multivariate analysis on the relationship between the hygiene-specific variables and the udder health variables on 144 farms are listed in Table 6. From all hygiene-specific variables described above, only the variables listed in Table 6 significantly ( $P \leq 0.05$ ) contributed to the fit of the model. There were no significant differences found in the udder health variables that were attributable to the different brand of the AMS used on the farm. In the final model the proportion of cows with dirty teats before milking and the proportion of cows with dirty thighs were positively related to annual average herd SCC. The squared correlation coefficient of this model was 0.10, so 10% of the variance in annual average herd SCC was explained by the 2 hygiene-specific variables. Annual average percentage NHSCC was positively related again to the proportion of cows with dirty teats before milking and to the proportion of milkings where teats were not covered with teat disinfecting spray after milking. The time interval between the milk production test days showed no significant relation with annual average percentage NHSCC. This model explained 10.6% of the variance in annual average percentage NHSCC. Annual incidence rate of CM was positively related to the frequency of replacing the milking filters. This hygiene-specific variable explained 6.0% of the variance in CM.

After Bonferroni correction, the only significant difference ( $P \leq 0.05$ ) in annual incidence rate of CM was seen between farms where the milking filters were replaced 3 times daily compared with farms where the milking filters were replaced once daily or less. Backward calculation showed that annual incidence rate of CM was 2.11 times higher on the farms where the milking filters were replaced 3 times daily compared with once daily or less.

The hygiene-specific variables in data set 2 and some of their interactions, as described earlier, are used for multivariate analysis at cow level. Results of this analysis on the relationship between cow hygiene scores and CSCC on 2,294 cows are listed in Table 7. Only the hygiene score of the udder significantly ( $P \leq 0.05$ ) contributed to the fit of the model and is listed on the left side in Table 7. The number of days between the farm visit and the milk production test day and the interaction term with the hygiene score of the udder showed no significant relation with CSCC.

Only the comparisons that significantly ( $P \leq 0.05$ ) contributed to the fit of the model after Bonferroni correction are listed in on the right side in Table 7. Cow SCC was significantly higher when cows had an udder hygiene score of 3 or 4 compared with an udder hygiene score of 2. Backward calculation showed that CSCC was 1.19 times higher for cows with an udder hygiene score of 3 compared with cows with an udder hygiene score of 2. Cow SCC was 1.38 times higher for cows with an udder hygiene score of 4 compared with cows with an udder hygiene score of 2.

## DISCUSSION

In this study the information on the farms was collected by a team of 8 different observers. The effect on repeatability was not measured. The students visited the farms in teams of 2, of which always 1 student was involved in the study as a part of their own research internship. All the students were at the end of their veterinarian science education and well trained in the different scoring parts. This should have had a positive influence on the repeatability between the observers. Reneau et al. (2005) used hygiene scores collected using a scorecard. This scorecard provided short descriptions



**Table 7.** Final general linear mixed model for cow SCC at cow level<sup>1</sup>

Variable	Cow SCC			Cow SCC after Bonferroni correction		
	$\beta$	SE	<i>P</i> -value	$\beta$	SE	<i>P</i> -value
Intercept	11.785	0.069	<0.001	11.462	0.054	<0.001
Hygiene score of the udder <sup>2</sup>						
1	-0.286	0.148	0.054	NR <sup>3</sup>	NR	NS
2	-0.323	0.078	<0.001	Ref <sup>4</sup>	—	—
3	-0.152	0.073	0.037	0.171	0.060	0.026
4	Ref	—	—	0.323	0.079	<0.001

<sup>1</sup> $\beta$  = estimated regression coefficient.

<sup>2</sup>1 = completely free of or has very little dirt; 2 = slightly dirty; 3 = mostly covered in dirt; 4 = completely covered, caked-on dirt.

<sup>3</sup>NR = not relevant.

<sup>4</sup>Ref = reference category.

of the areas to be scored and used simple drawings of each body area to convey the degree of cleanliness associated with scores. They found a high mean correlation coefficient (0.8) for hygiene scores in 5 categories assigned by 14 students and 2 faculty members. This indicates that the possible differences in hygiene scoring are not of great influence on the results found. Another part of the data collection was performed with a partially open-ended questionnaire. This approach was used so as to not force the farmers into specific answers, but sometimes this made it difficult to categorize the responses. Because of this, some information could not be used for further analysis. If the students would have been trained in conducting a questionnaire, there probably would have been fewer missing values. Variables with more than 5 missing values were not included in the final model. Therefore, we could have missed important variables. However, all hygiene-specific variables were included in the univariate analysis, and none of these variables with missing values were significantly related to the udder health variables. The annual incidence rate of CM was based on the information given by the farmer in the questionnaire. This makes this udder health variable less accurate than the other 2 measured udder health variables. Moreover, all the answers to the questions in the questionnaire were reported by the farmer, which is almost always less accurate than measurements.

The arithmetic mean of the annual average herd SCC in this research was 267,000 cells/mL. This is high compared with the average BMSCC of 192,000 cells/mL found in a recent study in the Netherlands by Jansen et al. (2009). Selection bias could be an explanation for the different results because the effect of nonresponding farmers could not be investigated. A previous study by Valeeva et al. (2007) showed that the responding farmers represented the entire population very well. Therefore, probably no selection bias has occurred, but an actual difference between the nationwide average SCC and the

average SCC on farms with an AMS exists. This is in accordance with previous studies (e.g., Van der Vorst and Hogeveen, 2000; Rasmussen et al., 2002).

Annual average percentage NHSCC provides information about the dynamics of udder health. Annual average percentage NHSCC was positively associated with the proportion of cows with dirty teats, so this is probably predisposing for obtaining new infections. The other hygiene-specific variable related to annual average percentage NHSCC was the proportion of milkings where the teats were not covered with teat disinfecting spray. On average, in 18% of the milkings the teats were not covered with teat disinfecting spray at all after milking. Besides this, there were also milkings where a small amount of the surface of the teats was covered with teat disinfecting spray after milking. Post-milking teat disinfection has shown to be an effective preventive measure for new intramammary infections (e.g., Lam et al., 1997). The association of no coverage of the teats with teat disinfecting spray with annual average percentage NHSCC can therefore be very well explained. The spraying system of the AMS should be improved.

Annual incidence rate of CM was significantly higher on farms where the milking filters were replaced 3 times daily compared with once or less daily. One explanation for this could be that the relation is reversed. A possibility is that when a farmer is experiencing a high incidence of CM, he makes more effort in preventive measures and one of them could be a more frequent replacement of the milking filters. Another explanation involves the detection of CM. When the farmer is replacing the milking filters more frequently, he might pay more attention to the clots on the filter, so he is experiencing a higher incidence of CM.

In both the analysis at farm level and the analysis at cow level, a direct relationship was found between cow hygiene and SCC. At farm level, the proportion of cows with dirty thighs and the proportion of cows

with dirty teats before milking were positively related to annual average herd SCC. The average percentage of cows with dirty teats before milking was 31%. After cleaning, 8% of the cows still had dirty teats, so the cleaning efficiency can still be improved. For example, a farm with high proportions of both cows with dirty teats and cows with dirty thighs had an estimated annual average herd SCC of 290,000 cells/mL. A farm with low proportions of both cows with dirty teats and cows with dirty thighs had an estimated annual average herd SCC of 212,000 cells/mL. This is a difference of 78,000 cells, which indicates that hygiene is very important for udder health. A more thorough udder cleaning by the milker might compensate for dirty udders. The technical performance of the AMS is the same for every milking. No adjustments on the cleaning procedure can be made for individual cows. Therefore, hygiene might be even more important on farms milking with an AMS. There are no comparable numbers available on conventional farms. For that reason, hygiene and hygiene management cannot be proven to be more important on farms with an AMS than on farms with a conventional milking system.

At cow level, the hygiene score of the udder was related to CSCC, with significant differences seen for the contrasts between scores 2 and 3 and between scores 2 and 4. According to the fitted model, a cow with an udder hygiene score of 2, 3, and 4 had an estimated CSCC of 95,000, 113,000, and 131,000 cells/mL, respectively. The hygiene scores of the legs were in none of the analyses related to SCC. Similar results were found in an earlier study on conventional farms. Schreiner and Ruegg (2003) found a linear relationship between linear SCS and udder hygiene score. For all contrasts of the udder hygiene scores, significant differences were observed except between scores 1 and 2 and between scores 3 and 4. In their results, a cow with an udder hygiene score of 2, 3, and 4 had an estimated CSCC of 102,000, 121,000, and 138,000 cells/mL, respectively, after transformation of linear SCS into SCC (Schukken et al., 2003). This is almost equal to the results found in this study. The relationship between leg hygiene score and linear SCS found by Schreiner and Ruegg (2003) was weak. In contrast, Reneau et al. (2005) found a stronger relationship between lower leg hygiene scores and linear SCS than between udder hygiene scores and linear SCS. The strongest relationship they found was the association between the udder-lower leg composite hygiene score, which was the average of both the separate scores, and linear SCS. Each 1-unit change in udder-hind limb composite hygiene score is associated with a change in bulk tank milk SCC of 40,000 to 50,000 cells/mL. The average cow SCC in that study

was over 400,000 cells/mL, which makes comparison with the results in the present study less easy.

The association between hygiene and udder health is clearly seen in the results, but some interference from management styles could also have occurred. Management style and BMSCC are related. Research showed that farmers of herds with a low BMSCC worked precisely rather than fast, and farmers of herds with a high BMSCC worked fast rather than precisely. Also, the hygiene on farms with cows with a low BMSCC was better than that on farms with cows with a high BMSCC (Barkema et al., 1999). Although management style could be confounded with hygiene-specific risk factors for poor udder health, a direct relationship is plausible considering the size of the effects found in this study. Previous studies also support the relationship between hygiene and udder health (Schreiner and Ruegg, 2003; Reneau et al., 2005).

Keeping lactating dairy cows as clean as possible before milking is important for good udder health, as was shown in this study. Exposure to dirt in the environment will probably affect cow hygiene. For example, Ward et al. (2002) found that bedding management, like type of bedding material and stocking, can cause herd differences in hygiene scores. A further analysis, which we did not present here, on the relation between environmental hygiene and cow hygiene based on data used in the present study showed comparable results. Cleaning frequency of the teat cups, bedding material, refill of fresh bedding material, removal of wet spots, calving area used as hospital, and cleanliness of the teat cup were significantly associated with cow hygiene. If there is a relationship between environmental hygiene and cow cleanliness, some relation between environmental hygiene and udder health is also expected. However, none of the environmental hygiene variables were significantly related to udder health in the different models. They are probably weakly related to udder health separately, but together they do affect udder health. To assess the hygiene of a farm to see whether improvements can be made, measuring cleanliness of the cows is more appropriate than measuring hygiene in the environment.

## CONCLUSIONS

Compared with farms with a conventional milking system, the risk of decreased udder health on farms with an AMS still appears to be present given the increased SCC values found in this study. The performed risk factor analysis confirms the relationship between cow hygiene and udder health on farms with an AMS. At the farm level as well as at the cow level, a direct

relation was seen between cow hygiene and SCC. No relation was found between the hygiene of the environment and udder health.

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## APPENDIX

Table A1. Variables in data set 1

Variable	n	Unit or category	Average or frequency <sup>1</sup>
Questionnaire: Farm characteristics			
Brand of the automatic milking system (AMS) <sup>2</sup>	144	Astronaut	86 (60)
		VMS	42 (29)
		Other (Galaxy, RMS, Merlin)	16 (11)
Questionnaire: AMS			
Cleaning frequency of the AMS space	144	Times/wk	9
Cleaning frequency of the laser or camera	144	Times/wk	13
Cleaning frequency of the milking tubes	144	Times/wk	12
Cleaning frequency of the teat cups	144	Times/wk	12
Cleaning frequency of the air inlets	144	Times/wk	3
Cleaning frequency of the robot arm	144	Times/wk	11
Cleaning frequency of the feeding trough	144	Times/wk	6
Cleaning frequency of the floor	144	Times/wk	12
Cleaning frequency of the teat cleaning system (roller brush system/cleaning cup)	144	Times/wk	6
Frequency of controlling the self-cleaning process of the AMS	142	Times/wk	1
Extra disinfection of the roller brush system	121	Yes	3 (2)
		No	118 (98)
Active substance of the disinfecting spray for the teat cleaning system	91	Hydrogen peroxide	79
		Chlorine	9
		Other	3
Frequency of controlling the amount of disinfecting spray for the teat cleaning system	81	Times/wk	2
Frequency of replacing the teat cleaning system (roller brush system or lining of the cleaning cup)	101	Times/wk	1
Frequency of controlling the premilking teat cleaning process	143	Times/wk	1
Teat spraying after milking	144	Yes	136 (94)
		No	8 (6)
Active substance of teat disinfecting spray	111	Chlorhexidine	13 (12)
		Linear benzenesulfonic acid	4 (4)
		Iodine	48 (43)
		Povidon-iodine	35 (31)
		Lactic acid	11 (10)
Frequency of controlling the amount of teat disinfecting spray in the AMS	110	Times/wk	4
Frequency of controlling the teat spraying process	141	Times/wk	1
Frequency of replacing the milking filters	144	≤1/d	38 (26)
		>1 ≤ 2/d	91 (63)
		3/d	15 (11)
Automatic replacement of the milking filters	144	Yes	17 (12)
		No	127 (88)
Moment of replacement of the milking filters	144	Always before cleaning	5 (3)
		Always after cleaning	18 (13)
		Other fixed intervals	101 (70)
		Not on fixed intervals	20 (14)
Questionnaire: Housing			
Ratio cubicle:cow	144		1.10
Ground layer of the cubicles	136	Mat	108 (80)
		Concrete	11 (8)
		Deep litter	15 (11)
		Water bed	2 (1)
Bedding material	144	Straw	28 (20)
		Sawdust	104 (72)
		None	5 (3)
		Other	7 (5)
Additions used on bedding material	144	Yes	22 (15)
		No	122 (85)
Storage of the bedding material	139	Silo	37 (27)
		Bags	102 (73)
Refill of fresh bedding material	144	<1/d	60 (42)
		≥1/d	84 (58)

*Continued*

**Table A1 (Continued).** Variables in data set 1

Variable	n	Unit or category	Average or frequency <sup>1</sup>
Removal of wet spots and manure from the cubicles	144	≤1/d	18 (12)
		2/d	73 (51)
		≥2/d	53 (37)
Flooring type	138	Closed	8 (6)
		Slatted	130 (94)
Automatic manure scraper	143	Yes	117 (82)
		No	26 (18)
Using frequency automatic manure scraper	85	Times/day	13
Nonautomated manure removal	144	Yes	95 (34)
		No	49 (66)
Separate calving area	144	With sight on the herd	100 (69)
		Without sight on the herd	24 (17)
		None	20 (14)
Calving area used as hospital	144	Yes	93 (65)
		No	51 (35)
Replacement of the bedding material of the calving area	129	Yes	71 (55)
		No	58 (45)
Disinfection of the calving area	134	Yes	33 (25)
		No	101 (75)
Questionnaire: Cow hygiene			
Shaving frequency of the cows	144	Times/yr	0.5
Shaving/flaming frequency of the udders	144	Times/yr	4
Shaving frequency of the tails	144	Times/yr	2
Percentage of cows leaking milk	135	%	6
Scoring protocol: Cleanliness AMS parts			
Cleanliness of the laser or camera	144	Clean	109 (76)
		Not clean	35 (24)
Cleanliness of the milking tubes	144	Clean	65 (45)
		Not clean	79 (55)
Cleanliness of the teat cup	144	Clean	85 (59)
		Not clean	59 (41)
Cleanliness of the air inlet	144	Clean	84 (58)
		Not clean	60 (42)
Cleanliness of the robot arm	144	Clean	81 (56)
		Not clean	63 (44)
Cleanliness of the feeding trough	144	Clean	60 (42)
		Not clean	84 (58)
Cleanliness of the floor	144	Clean	71 (49)
		Not clean	73 (51)
Cleanliness of the teat cleaning system (roller brush system/cleaning cup)	139	Clean	83 (58)
		Not clean	56 (42)
Scoring protocol: Functioning AMS			
Proportion of cows with score 3 (dirty) and 4 (very dirty) on cleanliness of the teats before milking	144		0.31
Average frequency of premilking teat cleaning process	143	Times/milking	1.5
Proportion of milkings where not all the teats were cleaned before milking	144		0.14
Proportion of cows with score 3 (dirty) and 4 (very dirty) on cleanliness of the teats after teat cleaning	139		0.08
Proportion of score 3 (poor) and 4 (bad) on self-cleaning process of the roller brush system	139		0.03
Proportion of milkings where teats were not covered with teat disinfecting spray	144		0.18
Proportion of milkings where milking cluster was not properly cleaned	144		0.04
Proportion of milkings where steam cleaning of the milking cluster was absent	144		0.84
Scoring protocol: Cow hygiene			
Proportion of cows with a dirty udder [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	144		0.94
Proportion of cows with dirty thighs [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	144		0.93
Proportion of cows with dirty legs [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	144		0.95

*Continued*

**Table A1 (Continued).** Variables in data set 1

Variable	n	Unit or category	Average or frequency <sup>1</sup>
Other			
Time interval between milk production test days	144	4 wk 6 wk	76 (53) 68 (47)
Udder health variables			
Annual average herd SCC	144	Cells/mL	269,000
Annual average percentage new cows with a high SCC	144	%	10
Annual incidence rate of clinical mastitis	144	Cases/100 cows/yr	28

<sup>1</sup>Values in parentheses are percentages.

<sup>2</sup>Astronaut: Lely Industries N.V. (Maassluis, the Netherlands); VMS: DeLaval International AB (Tumba, Sweden); Galaxy: SAC (Kolding, Denmark); RMS: GEA WestfaliaSurge BV (Zeewolde, the Netherlands); Merlin: Fullwood Limited (Shropshire, UK).

**Table A2.** Variables in data set 2

Variable	n	Category
Hygiene score of the udder	2,294	1 = completely free of or has very little dirt 2 = slightly dirty 3 = mostly covered in dirt 4 = completely covered, caked-on dirt
Hygiene score of the thighs	2,294	1 = completely free of or has very little dirt 2 = slightly dirty 3 = mostly covered in dirt 4 = completely covered, caked-on dirt
Hygiene score of the legs	2,294	1 = completely free of or has very little dirt 2 = slightly dirty 3 = mostly covered in dirt 4 = completely covered, caked-on dirt
Days between farm visit and milk production test day	2,294	≤7 d >7 d
Cow SCC	2,294	Cells/mL

**Table A3.** Selection of variables used for general linear models at farm level

Variable	Unit or category
Brand of the automatic milking system <sup>1</sup>	Astronaut VMS Other (Galaxy, RMS, Merlin)
Cleaning frequency of the teat cups	Times/wk
Cleaning frequency of the teat cleaning system (roller brush system/cleaning cup)	Times/wk
Frequency of replacing the milking filters	≤1/d >1 ≤ 2/d 3/d
Ratio cubicle:cow	—
Bedding material of cubicles	Straw Sawdust None Other
Refill of fresh bedding materials	<1/d ≥1/d
Removal of wet spots and manure from the cubicles	1/d 2/d ≥2/d
Nonautomated manure removal	Yes No
Separate calving area	With sight on the herd Without sight on the herd None
Calving area used as hospital	Yes No
Shaving/flaming frequency of the udders	Times/yr
Shaving frequency of the tails	Times/yr
Cleanliness of the teat cup	Clean Not clean
Proportion of cows with score 3 (dirty) and 4 (very dirty) on cleanliness of the teats before milking	—
Proportion of milkings where not all the teats were cleaned before milking	—
Proportion of milkings where teats were not covered with teat disinfecting spray	—
Proportion of cows with a dirty udder [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	—
Proportion of cows with dirty thighs [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	—
Proportion of cows with dirty legs [hygiene score 2 (slightly dirty), 3 (dirty), and 4 (very dirty)]	—
Annual average herd SCC	Cells/mL
Annual average percentage new cows with a high SCC	%
Annual incidence rate of clinical mastitis	Cases/100 cows/yr

<sup>1</sup>Astronaut: Lely Industries N.V. (Maassluis, the Netherlands); VMS: DeLaval International AB (Tumba, Sweden); Galaxy: SAC (Kolding, Denmark); RMS: GEA WestfaliaSurge BV (Zeewolde, the Netherlands); Merlin: Fullwood Limited (Shropshire, UK).