J. Dairy Sci. 86:3460–3465

Relationship Between Udder and Leg Hygiene Scores and Subclinical Mastitis

D. A. Schreiner[1] and P. L. Ruegg
Department of Dairy Science
University of Wisconsin, Madison 53706

ABSTRACT

The objective of this study was to determine the relationship between udder and leg hygiene scores of lactating dairy cattle and measures of subclinical mastitis. Study animals (n = 1250) consisted of lactating dairy cows from eight commercial dairy farms. Herds were enrolled during December 2000 and January 2001 and were visited bimonthly for a total of five visits per herd. Udder and leg hygiene scores were recorded by one person using a four-point scale ranging from one (very clean) to four (very dirty). Udder and leg hygiene scores were compared to bacteriological cultures of milk samples and monthly individual SCC values. Mean hygiene scores were 2.09 and 2.33 for udders and legs, respectively. Udder hygiene scores (UHS) were significantly associated with leg hygiene scores and varied among farms. Linear somatic cell scores increased as udder hygiene score increased. Significant differences in somatic cell scores were observed for all contrasts of udder hygiene score, except between scores of 1 and 2 and of 3 and 4. Linear somatic cell scores were associated with leg hygiene scores, but the only significant contrast was between leg hygiene scores of 2 and 4. There was a significant association between the prevalence of intramammary contagious pathogens and udder hygiene score. The prevalence of intramammary environmental pathogens was significantly associated with udder hygiene score and was 7.7, 10.0, 10.6, and 13.5% for UHS of 1, 2, 3, and 4, respectively. The prevalence of environmental pathogens was not associated with LHS. Cows with udder hygiene scores of 3 and 4 were 1.5 times more likely to have major pathogens isolated from milk samples compared with cows with hygiene scores of 1 and 2.
(**Key words:** mastitis, milk quality, somatic cell count, udder hygiene)

Received April 3, 2003.
Accepted May 14, 2003.
Corresponding author: P. L. Ruegg; e-mail: plruegg@wisc.edu.
[1]Present address: University of Wisconsin, Cooperative Extension, Green Lake Courthouse, 492 Hill St., Green Lake, WI 54941.

Abbreviation key: LHS = leg hygiene score, **UHS** = udder hygiene score.

INTRODUCTION

Exposure to mastitis pathogens and the efficiency of the bovine defense mechanism are two key factors that determine the risk of IMI (Hamann, 1991). Exposure can originate from several sources, including the environment of the cow, existing IMI, and teat skin flora (Pankey et al., 1987). Cleanliness of the udder is thought to influence the quantity and type of bacteria present on teat surfaces, and dirty teats and udders are considered to be a source of environmental bacteria in milk (Galton et al., 1982; Guterbock, 1984). In one study, the incidence of IMI was correlated with the number of mastitis pathogens present on the teat end (Neave et al., 1966). The quality of premilking udder preparation is an important determinant of milk quality. Premilking udder preparation must be efficiently performed because thorough preparation has been reported to increase the amount of time spent in the milking parlor (Smith et al., 1998). In a review article, Pankey (1989) reported that bacterial numbers in milk increase when teats are inadequately cleaned and dried. He also reported that the incidence of IMI is highly associated with the number of mastitis pathogens present on the teat end. In one study, herd management and animal hygiene were reported to have more influence on bulk tank SCC than dry cow therapy (Bodoh et al., 1976). Exposure to manure in cow housing areas can influence the rate of clinical mastitis. Bartlett et al. (1992) were able to predict the occurrence of clinical coliform mastitis using an index of environmental sanitation. Hygiene scoring systems have been used to assess the cleanliness of cows and the farm environment (Bartlett et al., 1992; Barkema et al., 1998, 1999; Ward et al., 2002; Reneau et al., 2003). The environment and the cows themselves were cleaner for herds that produced milk with lower SCC values compared with herds with higher bulk tank SCC values (Barkema et al., 1998). Farms with management styles characterized as “quick and dirty” were found to have higher bulk milk SCC values compared with farms with management styles characterized as “clean and accu-

rate" (Barkema et al., 1999). The SCC of cows with cleaner udders and lower rear legs was lower than SCS of cows with dirtier udders and legs (Reneau et al., 2003). The relationship between individual cow hygiene scores and IMI has not been reported. The objective of this study was to determine the relationship between udder and leg hygiene scores of lactating dairy cattle and measures of subclinical mastitis.

MATERIALS AND METHODS

Animals

Data were obtained from herds enrolled in another study, and herd selection criteria and demographic data have been previously described (Schreiner and Ruegg, 2002). Healthy, lactating multiparous and primiparous animals (n = 1250) from eight commercial dairy farms located in Wisconsin were enrolled. The number of lactating cows per herd ranged from 65 to 326 animals. All animals were housed in free stalls and were milked in parallel or herringbone parlors. Herds were required to be enrolled in an official DHIA program, have a bulk tank SCC of less than 500,000 cells/ml, and produce more than 20 kg of milk per cow per day at the beginning of the study. All housing and management decisions were the responsibility of the farmer. All herds were enrolled between December 2000 and January 2001, and data were collected for 8 to 9 mo.

Sample Collection and Analysis

Herds were visited on a bimonthly schedule for a total of five visits per herd. During each visit, university personnel collected composite milk samples from all lactating cows after premilking cow preparation and before unit attachment. Collection and microbiological procedures were defined and performed as outlined by the National Mastitis Council (1999). Milk samples were immediately put on ice and frozen upon arrival at the laboratory. In brief, thawed milk samples were streaked on one quarter of blood agar plates using 0.1-ml disposable plastic loops and incubated at 37°C for 24 to 48 h. The morphology and hemolysis patterns of bacterial colonies were determined, and significant organisms were differentiated using standard microbiologic methods. *Staphylococcus aureus* was identified using mannitol and coagulase reactions; *Streptococcus agalactiae* were identified using the CAMP test, esculin reactions, and agglutination; and gram-negative bacteria were tested using MacConkey agar, motility, indole, and ornithine reactions, and triple sugar iron slants.

The rate of IMI was determined for each of the five occasions when the entire herd was cultured. Milk samples were coded as negative (no growth), contagious pathogen (*Staphylococcus aureus*, *Streptococcus agalactiae*), environmental pathogen (*Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., *Enterococcus* spp.), minor pathogen (coagulase-negative *Staphylococcus* spp., *Actinomyces* spp., and *Corynebacteria* spp.) or contaminated (any culture with more than two bacterial species per sample unless *Strep. agalactiae*). For some statistical analyses, environmental and contagious pathogens were combined and categorized as major pathogens. Results from contaminated samples were not included in statistical analysis.

Individual cow SCC data were downloaded from DHIA for all available sample months for each farm.

Udder and leg hygiene scores were assessed during milk sample collection in the milking parlor using a previously described method (Schreiner and Ruegg, 2002). Udder and lower legs of study animals were compared to model animals depicted in photos on the scoring sheet and given a score based on the following categories: 1) Completely free of or has very little dirt, 2) slightly dirty, 3) mostly covered in dirt, or 4) completely covered, caked-on dirt. Scores were recorded and determined by one individual throughout the entire study. Consistency within observer was assessed by duplicate scoring of 100 lactating dairy cows not enrolled in the study. Duplicate scoring was performed immediately upon completion of the first scoring process. The assessor did not have access to the initial scores during the second scoring.

Statistical Analysis

Repeatability of hygiene scores and agreement between udder and leg scores were assessed using Kappa statistics (Martin et al., 1987). The relationship between UHS and LHS was analyzed using chi-square analysis (SAS, 1999). The relationship between SCS and prevalence of IMI was analyzed using chi-square analysis (SAS, 1999). Data were analyzed in a model that included effects of subject (animal), period (sample rounds 1, 2, 3, 4, and 5), SCS, and prevalence of IMI (yes or no). Linear score was grouped into categories for chi-square analysis (<1, 1 to 1.9, 2 to 2.9, 3 to 3.9, 4 to 4.9, 5 to 5.9, 6 to 6.9, 7 to 7.9, ≥8). The relationship between SCS and hygiene scores was analyzed using PROC MIXED analysis for repeated measures (SAS, 1999). Data were analyzed in a model that included effects of subject (animal), udder and leg hygiene scores (score of 1, 2, 3, or 4), period (sample rounds 1, 2, 3, 4, and 5), farm (n = 8), and linear score. The relationship between the prevalence of IMI and UHS and LHS were analyzed using chi-square analysis (SAS, 1999). Data were analyzed included effects of subject (animal), udder and leg cleanliness ranking (score of 1, 2, 3, or 4), and presence of infection (yes or no).

Table 1. Descriptive statistics by farm for herds enrolled.

Farm	Breed[1]	Number of cows enrolled	Bulk tank SCC (×1000)	Log SCC of enrolled cows		Prevalence of IMI		Hygiene score		% of Udder scores 3 or 4	% of Leg scores 3 or 4
					95% CI	Major[2]	Minor[3]	Udder	Leg		
1	H	67	122	1.78	1.62–1.95	6%	36%	1.88^{ab}	2.22^{bc}	17.39%	23.19%
2	H	71	261	1.95	1.80–2.10	16%	3%	2.22^{de}	2.60^{e}	28.46%	44.19%
3	H	130	412	2.41	2.29–2.53	31%	39%	2.40^{f}	2.71^{f}	35.31%	56.18%
4	H	110	319	2.01	1.87–2.15	16%	27%	1.98^{c}	2.18^{ab}	19.95%	24.47%
5	H	178	284	1.89	1.81–1.97	14%	24%	1.92^{bc}	2.22^{b}	14.85%	24.47%
6	J	184	221	2.13	2.05–2.21	22%	45%	2.28^{e}	2.29^{c}	28.91%	25.59%
7	H	184	128	1.84	1.76–1.92	9%	24%	1.84^{a}	2.13^{a}	12.63%	16.07%
8	H	326	324	2.06	2.00–2.12	13%	46%	2.16^{d}	2.41^{d}	21.96%	34.96%

$^{a–f}$Farms with different superscripts differ significantly ($P < 0.05$).

[1]H = Holstein, J = Jersey.

[2]*Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., and *Enterococcus* spp.

[3]Coagulase-negative *Staphylococcus* spp., *Actinomyces* spp., and *Corynebacteria* spp.

Udder and leg hygiene scores were categorized as "clean" (score of 1 and 2) or "dirty" (score of 3 or 4). The odds of isolation of major and minor pathogens based on hygiene category were calculated.

RESULTS

Animals

Descriptive characteristics of the enrolled herds have been previously reported (Schreiner and Ruegg, 2002). The mean (SE) parity and DIM at enrollment for enrolled animals (n = 1250) were 2.3 (0.04) and 114.1 (3.3), respectively. The mean milk yield and SCC at enrollment were 25.7 (0.41) kg and 235,636 (18,923) cells/ml, respectively. At the end of the study period, 157 (12.5%) of cows had been culled.

The prevalence of IMI caused by contagious pathogens was significantly different among farms, ranging from 0 to 21% of sampled animals ($P < 0.001$). The prevalence of environmental pathogens was significantly different among farms, ranging from 3.8 to 17.6% of sampled animals ($P < 0.001$). A large proportion of minor pathogens were recovered from the composite milk samples, and the prevalence of minor pathogens was significantly different between farms ranging from 24 to 46% of sampled animals ($P < 0.001$). Log SCC for enrolled cows varied significantly among farms ($P < 0.001$) as expected, given the differences in IMI (Table 1).

Hygiene Scores

Duplicate scoring of cows by a single observer indicated a high degree of repeatability of hygiene scoring (Table 2). The greatest agreement within observer occurred when scores were combined into categories of clean or dirty. Less agreement occurred between UHS and LHS.

Udder and leg hygiene scores varied significantly ($P < 0.01$) among farms (Table 1).

Mean UHS (n = 4695) and LHS (n = 4695) were 2.09 (0.80) and 2.33 (0.73), respectively. The distribution of hygiene scores was 20.4 and 6.4% (score 1); 58.0 and 63.7% (score 2); 14.2 and 20.4% (score 3); and 7.4 and 9.5% (score 4) for UHS and LHS, respectively. Udder hygiene scores were significantly associated with LHS ($P < 0.001$). Mean UHS of 1, 2, 3, and 4 had corresponding mean LHS of 1.86, 2.24, 2.70, and 3.42, respectively.

Subclinical Mastitis

Individual cow SCS data were collected from DHIA within an interval of 1 to 27 d of obtaining hygiene scores (mean interval was 8.5 d; SD = 6.4). Linear SCS was significantly associated with isolation of contagious pathogens ($P < 0.001$), environmental pathogens ($P < 0.001$), and minor pathogens ($P < 0.001$). The prevalence of major pathogens increased with the SCS until the SCS reached 6, and then it stabilized (Figure 1). Linear SCS increased with UHS ($P < 0.001$) (Table 3). Signifi-

Table 2. Agreement within observer and between first (A) and second (B) score for duplicate hygiene scores obtained from 100 cows.

Contrast[1]	Observed proportion agreement	Kappa
UHS A to UHS B	77%	0.57
LHS A to LHS B	85%	0.71
UHS A to LHS A	59%	0.25
UHS B to LHS B	70%	0.45
Clean vs. dirty[1]		
Udder scores	95%	0.71
Leg scores	96%	0.88

[1]UHS = udder hygiene score; LHS = leg hygiene score.

[2]Clean is defined as hygiene score 1 or 2, dirty is defined as hygiene score of 3 or 4.

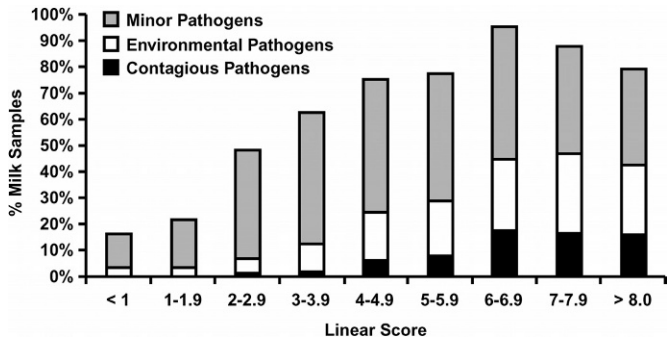

Figure 1. Distribution of isolation of contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*), environmental pathogens (*Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp., and *Enterococcus* spp.), and minor pathogens (coagulase-negative *Staphylococcus* spp., *Actinomyces* spp., and *Corynebacteria* spp.) from composite milk samples by linear SCC.

cant differences in SCS were observed for all contrasts of UHS except between UHS of 1 and 2 and UHS of 3 and 4 (Table 3). Linear SCS were associated with LHS ($P = 0.01$), but the only significant contrast was between LHS of 2 and 4 (Table 3).

There was a significant association between the prevalence of contagious pathogens and UHS ($P = 0.006$). The prevalence of IMI caused by contagious pathogens was 2.8, 4.7, 5.1, and 7.4% for UHS of 1, 2, 3, and 4, respectively. There was no significant relationship between prevalence of contagious pathogens and LHS ($P = 0.151$). The prevalence of IMI caused by contagious pathogens was 2.9, 4.2, 5.5, and 5.6% for LHS of 1, 2, 3, and 4, respectively.

The prevalence of environmental pathogens was significantly associated with UHS ($P = 0.046$). The prevalence of IMI caused by environmental pathogens was 9.7, 9.6, 12.1, and 13.8% for UHS of 1, 2, 3, and 4, respectively. The prevalence of environmental pathogens was not associated with LHS ($P = 0.151$). The prevalence of IMI caused by environmental pathogens was 7.7, 10.0, 10.6, and 13.5% for LHS of 1, 2, 3, and 4, respectively.

The prevalence of isolation of minor pathogens was independent of UHS and ranged from 34.8 to 37.8% ($P = 0.835$). The prevalence of isolation of minor pathogens was independent of LHS and ranged from 34.3 to 36.4% ($P = 0.751$).

Cows with UHS or LHS categorized as dirty were more likely to have major pathogens isolated from composite milk samples compared with cows with UHS or LHS categorized as clean (Table 4). There was no significant association between category of hygiene scores and isolation of minor pathogens from composite milk samples (Table 4).

Table 3. Linear score comparison between udder and leg hygiene score categories.

Hygiene score	Linear SCS	Contrast[1]			
		1	2	3	4
Udder					
1	2.93		0.09	0.33**	0.52***
2	3.03			0.24*	0.43**
3	3.27				0.19
4	3.46				
Leg					
1	3.13		–0.13	0.03	0.19
2	3.00			0.15	0.32**
3	3.15				0.17
4	3.32				

[1]Difference in mean linear score between hygiene scores.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

DISCUSSION

Key strategies for mastitis control must include effective methods to prevent the development of new infections and to eliminate existing infections (Neave, 1966). The presence of mastitis pathogens on teat ends has been correlated with the incidence of IMI (Pankey, 1989). Moisture, mud, and manure present in the environment of the cow are the primary sources of exposure for environmental mastitis pathogens, and hygiene scores of cows provide visible evidence of exposure to these potential sources. In this study, we were able to confirm the relationship between the measures of subclinical mastitis and measurements of animal hygiene.

The herds enrolled in this study were mid-sized commercial dairy farms that were reasonably representative of Wisconsin dairy herds that utilize free-stall housing. The SCC and rate of IMI varied considerably between farms, as did the proportion of hygiene scores that were categorized as dirty. All farms contained both clean and dirty animals, and, likewise, major and minor pathogens were isolated from milk samples obtained from all farms. Scoring was performed on individual animals and data were analyzed using cow as the experimental unit. Nevertheless, only eight herds contributed data to this study, and larger studies are needed to more precisely define the relationship between hygiene and mastitis.

The consistency of hygiene scoring within observer was assessed by duplicate hygiene scoring of the same animals and calculation of the kappa statistic (Martin et al., 1987). Kappa is used to compare agreement between tests and is calculated by subtracting the proportion of chance agreement from the proportion of observed agreement and dividing by the maximum possible agreement beyond chance. No agreement beyond chance results in a kappa of zero and kappa values of 0.4 to 0.5

Table 4. Prevalence of Pathogen by hygiene score.

	Hygiene score		
Isolation of pathogens	Clean[1]	Dirty[2]	Total
Udder hygiene scores			
Major pathogen			
No growth	1740	422	2162
Yes	474	171	645
Total	2214	593	2807
Proportion isolated	21.4%	28.8%	
χ^2		14.58	$P < 0.001$
Odds ratio	1.0	1.49	
95% CI		1.2–1.8	
Attributable fraction among exposed		32.8%	
Minor pathogen			
No growth	1740	422	2162
Yes	1217	331	1548
Total	2957	753	3710
Proportion isolated	41.1%	44.0%	
χ^2		1.94	$P = 0.164$
Odds ratio	1.0	1.12	
95% CI		0.95–1.32	
Attributable fraction among exposed		10.8%	
Leg hygiene scores			
Major pathogen			
No growth	1540	622	2162
Yes	424	221	645
Total	1964	843	2807
Proportion isolated	21.6%	26.2%	
χ^2		7.14	$P = 0.008$
Odds ratio	1.0	1.29	
95% CI		1.07–1.56	
Attributable fraction among exposed		22.5%	
Minor pathogen			
No growth	1540	622	2132
Yes	1094	454	1548
Total	2634	1076	3710
Proportion isolated	41.5%	42.2%	
χ^2		0.14	$P = 0.710$
Odds ratio	1.0	1.03	
95% CIl		0.89–1.19	
Attributable fraction among exposed		2.7%	

[1]Combined data for scores 1 and 2

[2]Combined data for scores 3 and 4.

indicate moderate agreement. Moderately high agreement was found for hygiene scores when all four levels of scores were included, but high levels of agreement were found when scores were categorized as either clean or dirty. Almost all differences in the duplicate scores were one level differences, and the system of hygiene scoring used in this study appears to be reasonably repeatable. Although there was a significant association between UHS and LHS, the agreement between these scores was fairly low.

Overall, 22% of UHS and 30% of LHS were categorized as dirty. A small proportion of animals received the lowest LHS (one indicating very clean), but 20% of UHS received this classification. Fecal consistency, bedding management, and stage of lactation have been previously suggested as contributing to herd differences in hygiene scores (Ward et al., 2002). The type of surface of the free-stall bed and the type of bedding used on that surface are likely to have a large influence on UHS but probably have less influence on LHS. Manure management systems, frequency of cleaning of barn alleys, and the ease of movement of the cattle are likely factors that have a larger influence on LHS than on UHS. Additional factors that could influence hygiene scores include overcrowding, dominance patterns among animals, and the number of times animals are moved for milking or management purposes.

As expected, SCS increased as the proportion of major pathogens isolated from milk samples increased. Linear SCS were significantly higher for UHS of 3 and 4, compared with UHS of 1 and 2, but no significant differences in SCS were observed within the categories of clean or dirty. Reneau et al. (2003) recently reported significantly increased SCS as hygiene scores of udders and lower

rear legs increased from 1 (clean) through 5 (very dirty). The herds (n = 9) included in that study appeared to have a higher prevalence of subclinical mastitis compared with the herds included in this study, as the mean SCC was 405,242 cells/ml. In our study, the relationship between LHS and SCS was weak. Whereas SCS were significantly associated with LHS, the only significant contrast was between LHS of 2 and 4.

The prevalence of isolation of both contagious and environmental mastitis pathogens from composite milk samples was significantly associated with UHS. The association between UHS and contagious mastitis was interesting and may indicate that control methods for contagious mastitis (e.g., teat dipping, sanitation) were not as effective when udders were dirty.

Animals with udders categorized as dirty were 1.5 times more likely to have major pathogens isolated from milk samples compared with animals with udders characterized as clean. Some IMI in the dirty group would likely have occurred due to other routes of exposure regardless of UHS. The attributable fraction in the exposed group is an estimate of the amount of IMI in the dirty group that is due to UHS. In this population of herds, more than one-third of IMI caused by major pathogens that occurred in the dirty cows could be attributed to UHS.

When examined separately over all hygiene scores, there was no significant association between LHS and the prevalence of IMI caused by environmental or contagious pathogens. When IMI were grouped together as major pathogens and hygiene scores were categorized as clean or dirty, animals with LHS categorized as dirty were 1.3 times more likely to have major pathogens isolated from milk samples than animals with LHS characterized as clean. These results may indicate that hygiene of legs is an important determinant of mastitis or may indicate that UHS are influenced by leg hygiene. Future research is needed to separate these effects, as this study was not designed for this purpose.

A number of studies have identified relationships between hygiene and milk quality, yet none of the studies directly correlated animal cleanliness with subclinical mastitis (Guterbock, 1984; Barkema et al., 1998; Khaitsa et al., 2000; Peeler et al., 2000). A study conducted in Great Britain found that the incidence of clinical mastitis was reduced on farms that had more strenuous sanitation and hygiene practice compared with farms with more relaxed practices (Peeler et al., 2000). Khaitsa et al. (2000) found that the type of premilking teat disinfection was significantly correlated to bulk tank SCC. More attention to hygiene practices was found in herds with lower SCC by Barkema et al. (1998).

Our study confirms the relationship between cleanliness of cows and the rate of subclinical mastitis. The hygiene scoring system used in this study was able to quantify relationships between udder and leg hygiene and the rate of IMI and linear SCC. An increased risk of IMI caused by major pathogens was identified for cows with udders characterized as dirty compared with udders characterized as clean.

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