



THE UNIVERSITY *of* EDINBURGH

Edinburgh Research Explorer

Cleanliness scores as indicator of *Klebsiella* exposure in dairy COWS

Citation for published version:

Munoz, MA, Bennett, GJ, Ahlstrom, C, Griffiths, HM, Schukken, YH & Zadoks, RN 2008, 'Cleanliness scores as indicator of *Klebsiella* exposure in dairy cows', *Journal of Dairy Science*, vol. 91, no. 10, pp. 3908-3916. <https://doi.org/10.3168/jds.2008-1090>

Digital Object Identifier (DOI):

[10.3168/jds.2008-1090](https://doi.org/10.3168/jds.2008-1090)

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Journal of Dairy Science

Publisher Rights Statement:

© American Dairy Science Association, 2008

General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



Cleanliness Scores as Indicator of *Klebsiella* Exposure in Dairy Cows

M. A. Munoz,¹ G. J. Bennett, C. Ahlström, H. M. Griffiths, Y. H. Schukken, and R. N. Zadoks²

Quality Milk Production Services, Cornell University, Ithaca, NY 14850-1263

ABSTRACT

This study was designed to explore the relationship between cow and udder cleanliness scores and the risk of isolation of *Klebsiella* spp. from lower hind legs and teat ends, respectively. The distribution of *Klebsiella* species was compared among isolates from teat ends, legs, and cases of clinical mastitis obtained from 2 dairy farms in New York State, with 850 and 1,000 cows, respectively. Farms were visited twice approximately 4 wk apart in August and September 2007 to obtain cleanliness scores and swabs from legs and teats. Isolates of *Klebsiella* clinical mastitis from each farm were collected from July through October 2007. Two studies were conducted. In the first study, whole-cow cleanliness of a purposive sample of 200 lactating cows was scored using a 4-point scale, and swabs were taken from their lower hind legs. In the second study, udder cleanliness of a separate convenience sample of 199 lactating cows was scored in the milking parlor, and swabs were taken from their teat ends before and after premilking udder preparation. Prevalence of *Klebsiella* spp. on legs and teat ends before udder preparation was 59 and 60%, respectively. Logistic regression was used to explore the association between isolation of *Klebsiella* spp. and cleanliness scores. Cow cleanliness scores and udder cleanliness scores were not associated with detection of *Klebsiella* on legs and on teats before udder preparation, respectively. After udder preparation, 43% of previously *Klebsiella* positive teat end samples remained positive, with significant differences between farms and months. Teats from dirty udders were significantly more likely to test positive for *Klebsiella* after udder preparation than teats from clean udders. The proportion of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates was similar for isolates from teat end swabs and clinical mastitis cases, supporting the notion that the presence of *Klebsiella* on teat ends may lead to opportunistic intramammary

infections. Udder cleanliness scores could be used as a management tool to monitor the risk of exposure to *Klebsiella* spp. on teat ends.

Key words: *Klebsiella*, cleanliness score, hygiene, dairy cow

INTRODUCTION

Coliform mastitis caused by *Klebsiella* spp. is a problem in New York State and elsewhere (Kikuchi et al., 1995; Munoz et al., 2007; Paulin-Curlee et al., 2007). *Klebsiella* mastitis occurs more frequently in herds that have a low bulk milk SCC than in herds with medium or high bulk milk SCC (Barkema et al., 1998). Severity of clinical episodes, poor response to vaccination, and the lack of effective treatments make *Klebsiella* especially troublesome compared with *Escherichia coli*, the other common cause of coliform mastitis (Erskine et al., 2002; Hogan and Smith, 2003; Roberson et al., 2004).

Identification of potential sources of *Klebsiella* is important for implementation of preventive measures that decrease exposure and limit the risk of udder infections. Bedding materials from wood byproducts such as sawdust and shavings can be sources of *Klebsiella* on dairy farms (Hogan et al., 1989; Zdanowicz et al., 2004). Moisture, organic matter content, and pH affect growth of *Klebsiella* in bedding (Thomas et al., 1983; Zdanowicz et al., 2004; Hogan et al., 2007). Fecal shedding of *Klebsiella* by about 80% of healthy dairy cows has been documented (Munoz et al., 2006; Munoz and Zadoks, 2007). Direct contact of the teat ends with materials that contain *Klebsiella*, such as bedding, feces, manure splash, water, milk, mattresses, legs, or milking machine liners may provide the bacteria with access to the udder, which may ultimately result in IMI (Bramley and Neave, 1975; DeHart et al., 1976). Clinical mastitis (CM) caused by *Klebsiella* can be related to high levels of environmental *Klebsiella* contamination (Carroll and Jasper, 1978) and to high *Klebsiella* counts in bedding (Hogan et al., 1989; Todhunter et al., 1991). Cows should be kept clean to reduce teat-end exposure to coliform organisms (DeHart et al., 1976). Evaluation of cow cleanliness provides an estimate of the degree of challenge to which dairy cows are exposed (Hughes, 2001) and can be used as a tool to monitor and control

Received February 11, 2008.

Accepted June 7, 2008.

¹Corresponding author: mam285@cornell.edu

²Current address: Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, UK.

Table 1. Description of herd management characteristics

Farm characteristic	Farm A	Farm B
Herd		
Size (milking cows)	1,000	850
Average daily milk production (kg)	35.0	37.3
Rolling bulk milk SCC (cells/mL)	363,500	377,500
Bedding and alley cleaning		
Type	Wood shavings	Sawdust
Replacement (times/2 wk)	7	4
Alley scraping (passes/3 h)	4	3
Milking		
Number of units	Double 16	Double 12
Crew members	2	2
Routine	Territorial, 5 or 6 cows/milker	Territorial, 6 cows/milker
Premilking teat disinfectant		
August	Peroxide 0.5%	Iodine 0.5%
September	Iodine 0.5%	Peroxide 0.5%

bacterial exposure levels (Bartlett et al., 1992; Ward et al., 2002; Reneau et al., 2005). Bacterial challenge on teats and premilking udder preparation protocols can affect bacterial counts in milk and new IMI rates (Galton et al., 1988; Magnusson et al., 2006). Previous field studies have established a significant association between udder and hind limb cleanliness scores of lactating dairy cattle, and measures of subclinical mastitis such as rate of IMI and linear SCS (Schreiner and Ruegg, 2003; Reneau et al., 2005). In a small-scale experimental study, however, no association was found between udder cleanliness scores and *Klebsiella* levels on teats before udder preparation (Zdanowicz et al., 2004).

The goal of our study was to expand on previous studies of cows' cleanliness scores and exposure to *Klebsiella* in areas that are close to the streak canal such as teat ends and (when the cow is lying down) the lower hind legs. Specifically, the aims were 1) to assess the association between overall cow cleanliness scores and the risk of isolation of *Klebsiella* spp. from cows' legs; 2) to assess the association between udder cleanliness score and the risk of isolation of *Klebsiella* from teat ends before and after premilking udder preparation; and 3) to compare the distribution of *Klebsiella* spp. isolated from teats, legs, and cases of CM. If cleanliness scores are an indicator of the risk of *Klebsiella* exposure, they could be used as management tools to aid in the prevention and control of *Klebsiella* mastitis.

MATERIALS AND METHODS

Animals and Farms

Two dairy farms were selected based on a history of *Klebsiella* mastitis problems, as determined using

bacteriological culture of milk samples from cows with clinical mastitis in the 12 mo preceding the study. Additional criteria included location (i.e., separate counties in northern New York State), herd size of >500 cows, and willingness to participate in the study. Farms were visited twice, approximately 4 wk apart, in August and September 2007. Farm A and farm B housed Holstein-Friesian herds in free-stall facilities with water-filled mattresses. Alley scraping on both farms was done using automatic scrapers. Alley scraping and bedding replacement frequencies did not change during the study. The premilking udder preparation routine, henceforth called udder preparation, for both farms consisted of forestripping, predipping, wiping with a cloth towel, and unit attachment. Farm B switched from forestripping followed by predipping in August to predipping followed by forestripping on September 4, on which day the milking crew received training. No changes in milking staff occurred at either farm during the study period. Additional management, herd, and milking characteristics for farms A and B are listed in Table 1.

Sample Collection and Cleanliness Scores

Two visits per farm were done, the first visit in the second week of August and the second visit in the second week of September 2007. Both farms were visited on the same day. Sample size calculation was performed to find a difference of 30% in the prevalence of *Klebsiella* between clean and dirty cows, with a power of 80% and α of 5%.

Study 1. During each farm visit, overall cow hygiene scores were determined for 50 cows. Because the goal of the study was to assess the association between cleanliness scores and *Klebsiella* detection, cows were

not selected at random. Purposive sampling of animals was used to obtain, as far as possible, approximately equal numbers of observations for each cleanliness score (Dohoo et al., 2003). For each cleanliness score, animals were selected from 4 or 5 lactating cow pens at each farm visit to avoid confounding by pen-specific associations. Cows were scored on a 4-point cleanliness scale, evaluating lower and upper hind legs, udder, and flanks (1 = very clean to 4 = very dirty). If all 3 body sites were free of soil, the cow was scored as 1; if some soil was present in any of the 3 sites, the cow was scored as 2; if at least 1 of the 3 sites was markedly soiled, the cow was scored as 3; if 2 or more sites were heavily soiled, the cow was scored as 4; non-peer-reviewed standard scorecard available online (<http://www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf>; Cook and Reinemann, 2007). Across farm visits, all cow cleanliness scores were determined by the same observer with the aid of the aforementioned standard scorecard. Immediately after scoring, each cow was sampled by swabbing the inner surface of both lower hind legs, using one individually wrapped 2 in. × 2 in. sterile gauze pad per cow (CVS Pharmacy Corp., Woonsocket, RI). Swabs were rubbed vigorously against the skin 4 or 5 times per leg, moving from top to bottom between the hock and fetlock joint.

Study 2. In the milking parlor, a convenience sample of 50 animals was evaluated. All cows in alternating milking rows were scored and sampled. For each cow, udder cleanliness score was recorded (1 = very clean to 4 = very dirty), using a peer-reviewed udder scoring system (Schreiner and Ruegg, 2002). Across farm visits, all udder cleanliness scores were determined by the same observer with the aid of a standard scorecard. Whole-cow scoring, done in the pens, was performed at the same time as the udder scoring, done in the milking parlor; therefore, cow cleanliness scores were determined by a different observer and for different animals than udder cleanliness scores. After udder cleanliness scoring, a single gauze pad was used per cow to sample all teat-ends before udder preparation. Subsequently, milkers performed udder preparation using the herd's standard procedure. After udder preparation all teat ends were swabbed a second time, using a second sterile gauze pad per cow. Gauze pads were rubbed vigorously against the skin of each teat end.

Immediately after sample collection, swabs from legs or teat ends were placed in individual sterile vials (Capitol Vial, Auburn, AL) containing 5 mL of Luria-Bertani broth (Difco LB Broth, Becton Dickinson and Co., Sparks, MD). Vials with swabs were kept cold and transported to the laboratory in cooler boxes with ice packs. Nitrile gloves were used throughout the sampling procedures.

Milk samples from CM cases were collected on both farms from July through October 2007. Milk samples were collected for 4 mo to obtain sufficiently high numbers of isolates to allow for meaningful comparison of the species distribution between isolates obtained from swabs and those obtained from animals with CM. The samples were aseptically collected (National Mastitis Council, 1999) by the herd managers. Frozen samples from farms A and B were picked up by Quality Milk Production Services (QMPS) personnel twice and once a week, respectively, and were transported in cooler boxes to the QMPS northern regional laboratory for microbiological analysis.

Laboratory Analysis

Gauze pads were incubated in Luria-Bertani broth for 4 h at 37°C, and approximately 0.01 mL of the enrichment was swabbed onto *Klebsiella* selective medium consisting of MacConkey agar no. 3 (CM0115, Oxoid Ltd., Basingstoke, UK) containing 10 mg/L of ampicillin (Munoz et al., 2006). The MacConkey plus ampicillin plates were incubated for 18 to 24 h at 37°C, and putative *Klebsiella* colonies were identified based on colony morphology (Munoz et al., 2006). Pink-yellow, mucoid, lactose-positive colonies were considered to be *Klebsiella* spp. and sub streaked onto trypticase soy agar with 5% sheep blood and 0.1% esculin (TSA-BE; PML Microbiologicals, Mississauga, Ontario, Canada) to obtain pure cultures. Identity of *Klebsiella* species was determined based on citrate utilization, motility test, and indole production (National Mastitis Council, 1999). Cultures on TSA-BE were inoculated on Simmons citrate agar slants using sterile 1- μ L loops, and stabbed into vials with motility-indole-ornithine medium with sterile culture needles. Citrate slants and motility-indole-ornithine vials were incubated at 37°C for approximately 16 to 48 h and 16 h, respectively. *Klebsiella pneumoniae* ATCC 13883 was used as a positive control for citrate utilization and *Escherichia coli* ATCC 25922 as negative control. For motility testing, *E. coli* ATCC 25922 was used as positive control and *K. pneumoniae* ATCC 13883 as negative control. The indole reaction was tested using the indole spot test (BBL DMACA indole reagent droppers, Becton Dickinson and Co.); *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 13883 were used as positive and negative controls, respectively. Indole-positive *Klebsiella* isolates were considered to belong to the species *K. oxytoca*, and indole-negative isolates were considered to belong to the species *K. pneumoniae*. Samples were considered to contain *Klebsiella* spp. if at least 1 colony from the cultured sample was confirmed to belong to 1 of the 2 species.

Milk samples from CM cases were processed using routine methods for the isolation and identification of mastitis pathogens (National Mastitis Council, 1999). Briefly, approximately 0.01 mL of each milk sample was swabbed onto a TSA-BE plate and growth was evaluated after 24 and 48 h of incubation at 37°C. Coliform organisms, including putative *Klebsiella* isolates, were identified based on colony morphology, subcultured onto MacConkey agar, and incubated overnight at 37°C. Species identification was performed as described for isolates from swabs. Cases of CM were considered to be caused by *Klebsiella* spp. if 2 or more *Klebsiella* colonies were found. If 3 or more bacterial species were cultured from a sample, the sample was considered contaminated.

Statistical Analysis

The proportion of swabs testing positive for *Klebsiella* spp. was compared between farm and months. The proportion of *Klebsiella* isolates belonging to the species *K. oxytoca* and *K. pneumoniae* was compared between sources on each of the farms. Differences in proportions were evaluated using Chi-square and Fisher's exact statistics. The reduction in the number of teats testing positive was calculated as [(% positive before udder preparation - % positive after udder preparation)/% positive before udder preparation]. Logistic regression was used to determine whether *Klebsiella* spp. isolation from legs was associated with cow cleanliness scores. *Klebsiella* isolation was the binary dependent variable, and independent variables were cleanliness score, farm, and month. A transition model was applied for *Klebsiella* isolation from teat swabs (Schukken et al., 2004), to assess factors influencing new and persistent *Klebsiella* presence on teat-ends after udder preparation. Isolation of *Klebsiella* post preparation was the dependent binary outcome. Statistical analyses were conducted with JMP version 7.0 (SAS Institute Inc., Cary, NC). Significance was declared at $P \leq 0.05$. The structure of the transition logistic regression model used for analyzing the data was

$$\text{Logit}(\text{Kleb}_{\text{after}}) = \text{Intercept} + \text{Kleb}_{\text{before}} + \text{score} \\ + \text{risk factor} + \text{Kleb}_{\text{before}} \times \text{risk factors} + \text{error},$$

where $\text{Kleb}_{\text{after}}$ is the *Klebsiella* isolation status after udder preparation and $\text{Kleb}_{\text{before}}$ is the *Klebsiella* isolation status before udder preparation. The intercept is the risk of isolating *Klebsiella* after udder preparation given that the pre-preparation sample was negative for *Klebsiella*. The sum of intercept and $\text{Kleb}_{\text{before}}$ is the risk of isolating *Klebsiella* after udder preparation

given that the pre-preparation sample was positive for *Klebsiella*. Score is the udder cleanliness score (3 levels: 1 = very clean; 2 = some visible contamination; 3+4 = dirty or very dirty). Farm and month were considered in the analysis as risk factors.

RESULTS

Isolation of *Klebsiella* spp. from Legs and Teats

Study 1. Across farms and months, 59.0% of swabs from legs tested positive for *Klebsiella* spp. For farm A, results were similar for both months. For farm B, isolation of *Klebsiella* spp. was more common from leg swabs obtained on the sampling day in August (98% positive) than from those obtained on the sampling day in September (22% positive; Table 2). Detection levels matched our impressions of farm hygiene at a qualitative level. Stall and alley hygiene was not noticeably different between August and September for farm A, whereas facilities and animals in farm B were considerably cleaner in September than in August. In August, overall hygiene on farm A appeared better than on farm B, whereas the reverse was true for September. On farm B, there were very few animals with cow cleanliness score 1 available for sampling in August, whereas animals with scores 2 and 3 were common. In September, few animals with cow cleanliness score 3 were available on farm B, but several animals with score 1 were observed. Animals with score 4 were rarely observed at any farm visit.

Study 2. Results for teat end swabs before udder preparation resembled results from leg swabs in that detection levels were similar in August and September for farm A; greatest for farm B in August; and lowest for farm B in September (Table 2). Overall, 59.8% of teat ends tested positive for *Klebsiella* spp. before udder preparation, and 31.2% of teat ends tested positive for *Klebsiella* spp. after udder preparation.

For each farm visit, the number of *Klebsiella*-positive swabs after udder preparation was significantly lower than the number of *Klebsiella*-positive swabs before udder preparation ($P \leq 0.01$), but udder preparation reduced the average proportion of *Klebsiella*-positive swabs by less than 50% (Table 2). The proportions of teat end swabs testing positive for *Klebsiella* did not differ between months for farm A ($P = 0.737$), whereas results differed significantly between months for farm B ($P = 0.000$). The proportions of *Klebsiella*-positive teat end swabs between farm A and farm B differed in August ($P = 0.000$), but not in September ($P = 0.062$). Most teat ends that tested positive for *Klebsiella* after udder preparation had also tested positive for *Klebsiella* before udder preparation (Table 3).

Table 2. Number (and proportion) of swabs from inner lower hind legs and clean (1+2) and dirty (3+4) teat ends, before and after premilking udder preparation, that tested positive for *Klebsiella* spp.

Farm/month	Legs	Udder cleanliness score (n) ¹	Teat ends		
			Before	After	Reduction ² (%)
Farm A					
August	28 (56.0)	1+2 (37) 3+4 (12)	24 (64.9) 5 (41.7)	5 (13.5) 6 (50.0)	79.2 -19.9
September	30 (60.0)	1+2 (46) 3+4 (4)	21 (45.7) 3 (75.0)	10 (21.7) 1 (25.0)	52.5 66.7
Farm B					
August	49 (98.0)	1+2 (34) 3+4 (16)	34 (100) 16 (100)	23 (67.6) 14 (87.5)	32.4 12.5
September	11 (22.0)	1+2 (39) 3+4 (11)	14 (35.9) 2 (18.2)	2 (5.1) 1 (9.1)	85.8 50.0
Total	118 (59.0)		119 (59.8)	62 (31.2)	47.8

¹Number of observations in each score category.

²Percentage of reduction in *Klebsiella* prevalence on teat ends after udder preparation: [(% positive before udder preparation - % positive after udder preparation)/% positive before udder preparation].

Cleanliness Score and *Klebsiella* Isolation

The probability of detection of *Klebsiella* in swabs obtained from the lower hind legs did not differ significantly between “clean” cows (cow cleanliness score 1 and 2; 53.4% positive) and “dirty” cows (cow cleanliness scores 3 and 4; 67.6% positive; $P = 0.059$), using whole-cow cleanliness scoring. In addition, no significant differences were found in the isolation of *Klebsiella* from legs with different cleanliness scores (3 levels: scores 1, 2, or 3+4; Figure 1, top panel) after correcting for the effect of farm and month ($P = 0.542$).

Swabs obtained from teats of “clean” udders before udder preparation (udder cleanliness scores 1 and 2; 59.6% positive) were equally likely to contain *Klebsiella* as swabs obtained from teats of “dirty” udders (udder cleanliness scores 3 and 4; 60.5% positive; $P = 0.920$). In addition, when correcting for farm and month, *Klebsiella* detection levels before udder preparation were not different ($P = 0.255$) across cleanliness scores

(scores 1, 2, or 3+4; Figure 1, middle panel). After udder preparation, *Klebsiella* was isolated more frequently from udders that had been classified as “dirty” before udder preparation (51.3%) than from udders that had been classified as “clean” (25.6%; $P = 0.001$). When correcting for farm and month, the proportion of *Klebsiella* positive samples after udder preparation was significantly greater for dirty udders ($P = 0.002$; scores 1, 2, or 3+4; Figure 1, bottom panel).

Modeling *Klebsiella* Presence on Teat Ends After Udder Preparation

Parameter estimates and odds ratios for the transition logistic regression model are presented in Table 4. Dirty udders were a significant risk factor for the presence of *Klebsiella* after udder preparation (odds ratio = 2.86; 95% confidence interval = 1.50 to 5.46). The risk of *Klebsiella* detection after udder preparation differed between herds and months. The baseline

Table 3. *Klebsiella* isolation from teat end swabs before and after premilking udder preparation¹

Farm/month	<i>Klebsiella</i> isolation ²				Total
	++	+-	-+	--	
Farm A					
August	7 (14.3)	22 (44.9)	4 (8.2)	16 (32.6)	49 (100)
September	6 (12.0)	18 (36.0)	5 (10.0)	21 (42.0)	50 (100)
Farm B					
August	37 (74.0)	13 (26.0)	0 (0.0)	0 (0.0)	50 (100)
September	1 (2.0)	15 (30.0)	2 (4.0)	32 (64.0)	50 (100)
Total	51 (25.6)	68 (34.2)	11 (5.5)	69 (34.7)	199

¹Numbers represent cows, with the proportion of cows in that category, as calculated within row, shown in parentheses.

²*Klebsiella* isolation: ++ = *Klebsiella* spp. isolated from swabs taken before and after udder preparation; +- = *Klebsiella* spp. isolated from swabs taken before but not after udder preparation; -+ = *Klebsiella* spp. isolated from swabs taken after but not before udder preparation; -- = *Klebsiella* spp. not isolated from swabs taken before or after udder preparation.

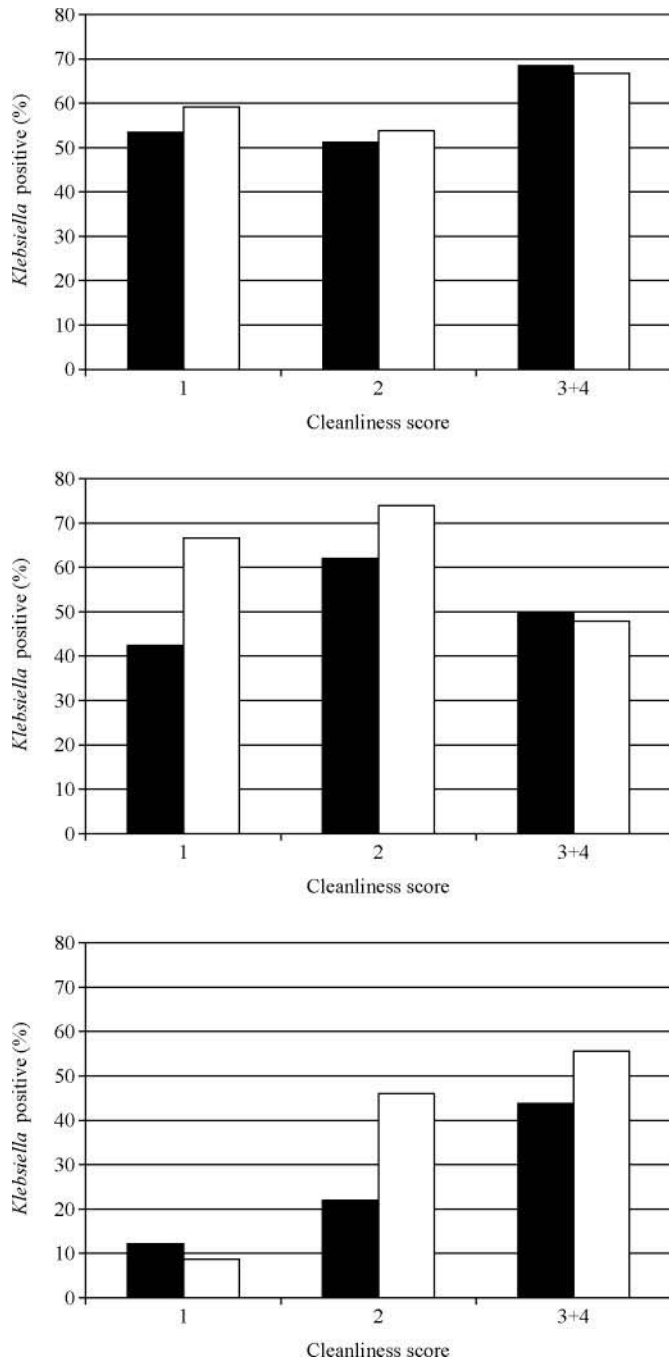


Figure 1. Proportion of cows, grouped by overall cow cleanliness score, testing positive for *Klebsiella* spp. on lower hind legs (top), and proportion of cows, grouped by udder cleanliness score, testing positive for *Klebsiella* spp. on teat-ends before (middle) and after (bottom) udder preparation. Black bars = farm A; white bars = farm B. Fifty observations were included per farm per month, with the exception of teat ends on farm A in August (49 observations).

risk for detection of *Klebsiella* after udder preparation, when correcting for cleanliness score, was based on farm A in September. In August, the risk of detection of *Klebsiella* after udder preparation was 2.1 times as great as in September for farm A. For farm B, the risk of detection of *Klebsiella* after udder preparation in August and September was 6.5 and 3.2 times as great, respectively, as the baseline risk for farm A in September. As shown in Table 4, detection of *Klebsiella* before udder preparation was not significant as a predictor for the detection of *Klebsiella* after udder preparation in the specified model. The combination of herd, month, and their interaction is a proxy for the probability of detection of *Klebsiella* before udder preparation. When farm, month, and their interaction were removed from the model (data not shown), the risk of *Klebsiella* detection after udder preparation was significantly associated with isolation of *Klebsiella* before udder preparation (odds ratio = 2.14; 95% confidence interval = 1.45 to 3.15; $P = 0.000$).

Distribution of *Klebsiella* Species from Legs, Teat Ends, and Clinical Mastitis

The species distribution of *Klebsiella* from legs and teat ends was compared with the species distribution of *Klebsiella* isolates from CM cases. The number of *Klebsiella* isolates available from CM cases each month from July through October were 7, 2, 6, and 0 for farm A (15 total), and 16, 8, 9, and 9 for farm B (42 total), respectively. On farm A, *K. pneumoniae* and *K. oxytoca* each constituted a considerable proportion of isolates from all sources (Table 5). On farm B, *K. pneumoniae* isolates outnumbered *K. oxytoca* isolates by a great margin. On farm B, species distribution was similar across sample types. On farm A, species distribution was similar for isolates from teat ends and CM cases (Table 5). The distribution of *Klebsiella* species among isolates from leg swabs differed from the distribution among isolates from CM cases and teat ends before and after udder preparation on farm A ($P = 0.004$).

DISCUSSION

Klebsiella mastitis is an increasingly important problem in dairy herds in New York State. Economic losses due to death of cows in the acute phase of infection or culling of cows with chronic infection can be considerable on individual farms. Because treatment and vaccination do little to prevent infection or limit the damage resulting from infection, prevention is of paramount importance in the control of *Klebsiella* mastitis. Dirty udders were found to be a significant risk factor for presence of *Klebsiella* after udder preparation. Our

Table 4. Results of logistic regression analysis of risk factors for the presence of *Klebsiella* spp. on teat ends after udder preparation on 2 dairy farms in northern New York State

Variable	Estimate	SE	OR ¹	95% Confidence limits for OR		P-value
Intercept	-1.15	0.24	—	—	—	<0.001
<i>Klebsiella</i> before udder preparation positive	0.12	0.24	1.13	0.70	1.80	0.609
Cleanliness score (baseline: score 1)						
Score 2	0.11	0.27	1.12	0.66	1.89	0.691
Score 3+4	1.05	0.33	2.86	1.50	5.46	0.001
Farm B (baseline: farm A)	0.08	0.22	1.08	0.70	1.67	0.707
Month August (baseline: September)	0.72	0.24	2.05	1.28	3.29	0.002
Farm B × month August	1.07	0.24	2.92	1.82	4.67	<0.001

¹OR = odds ratio.

results underscore the importance of applying teat disinfectant to udders without visible manure soiling. Even when udder preparation procedures include the use of teat disinfectants, they may not be sufficiently rigorous or effective to disinfect the teats of cows that enter the milking parlor with udders that would be classified as category 3 or above.

Coliform presence on teat ends after udder preparation is a source of exposure to pathogens during milking (DeHart et al., 1976). Our observations showed that the species distribution of *Klebsiella* isolates on teat ends was similar to the species distribution of *Klebsiella* isolates from CM cases and agrees with the idea that teat end contamination with *Klebsiella* may result in opportunistic IMI. Previously, udder cleanliness scores have been associated with IMI (Schreiner and Ruegg, 2003) and SCS (Reneau et al., 2005). Earlier attempts to correlate udder cleanliness scores to *Klebsiella* counts on teat ends did not reveal a clear association (Zdanowicz et al., 2004). Zdanowicz et al. (2004) evaluated *Klebsiella* counts on teats before but not after udder preparation. In agreement with their results, we did not find an association between udder cleanliness scores and *Klebsiella* prevalence before udder preparation. In contrast to Zdanowicz et al. (2004), we used an enrichment method and did not enumerate colonies within samples. At a qualitative level, we

noticed that for most sample pairs that tested positive before as well as after udder preparation, *Klebsiella* numbers were smaller in enrichments from swabs collected after udder preparation.

This study was limited to 2 herds in northern New York State, implying that generalization of results across herds elsewhere may not be justified. Results from the sampling in August, as well as culture results from samples collected from bedding and alleyways collected in June, July, and August (Zadoks et al., 2008) were shared with the producers that participated in the study. On farm B, this information was the incentive for management changes between August and September, including milker training and improved barn hygiene. The number of observations at herd level, however, was too small to attribute observed differences in the level of *Klebsiella* contamination between herds and months to herd or management factors in our study. Given our findings, further investigation of the role of herd management, udder preparation, and teat disinfectants in presence of *Klebsiella* on teats after udder preparation appears warranted.

Cleanliness of udders, flanks, and legs of dairy cows is associated with the consistency of feces (Ward et al., 2002). Although early studies showed that *Klebsiella* was uncommon in feces of dairy cows (Carroll and Jasper, 1978), recent studies showed that, on average,

Table 5. Number (and proportion) of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates from leg swabs, teat end swabs before and after udder preparation, and clinical mastitis cases

Sample	Farm A		Farm B	
	<i>K. pneumoniae</i>	<i>K. oxytoca</i>	<i>K. pneumoniae</i>	<i>K. oxytoca</i>
Legs	44 (75.9)	14 (24.1) ¹	56 (93.3)	4 (6.7)
Teat ends				
Before preparation	24 (45.3)	29 (54.7)	55 (83.3)	11 (16.7)
After preparation	10 (45.5)	12 (54.5)	36 (90.0)	4 (10.0)
Clinical mastitis	7 (46.7)	8 (53.3)	39 (92.9)	3 (7.1)

¹Proportion on legs was significantly different ($P = 0.004$) from teat ends before and after udder preparation and from mastitis isolates. No other significant differences were observed within farm ($P = 0.995$ for farm A, $P = 0.251$ for farm B).

80% of healthy adult dairy cows in New York State shed *Klebsiella* in feces during the summer (Munoz et al., 2006, 2007). In the herds that participated in the current study, fecal prevalence of *Klebsiella* was 67% (Zadoks et al., 2008). *Klebsiella* was found at similar prevalence (proportion of samples testing positive) on the cows' bodies; that is, on teats before udder preparation (60%) and on lower hind legs (59%; Table 2) and in the cows' environment; that is, in bedding (63%) and alleyways (66%; Zadoks et al., 2008). Our hypothesis that cow cleanliness might be used as an indicator of the probability of detection of *Klebsiella* on lower hind legs in individual animals was not supported by our data. Similarly, Schreiner and Ruegg (2003) found that the prevalence of IMI caused by environmental pathogens was not associated with leg hygiene scores. On the other hand, Reneau et al. (2005) reported that cleanliness scores of lower hind legs on a 5-point scale were significantly associated with SCS. We performed a cow cleanliness score using a non-peer-reviewed scoring system, which is partly reflective of leg cleanliness and less accurate. Whether lower hind limb cleanliness scores could be used as a tool to assess *Klebsiella* contamination of legs is therefore not yet clear, and neither is its relevance to the risk of exposure of udders to *Klebsiella*. Conversely, the use of a validated udder cleanliness score, if utilized consistently, can be a useful tool to estimate and reduce exposure of *Klebsiella* on teat ends after premilking udder preparation.

CONCLUSIONS

Premilking udder preparation was not sufficient to remove all *Klebsiella* from teat skin, especially when udders were dirty. The species distribution of *Klebsiella* isolates was similar for teat ends and CM cases, suggesting that opportunistic infections may result from teat end contamination. Udder hygiene scores can be used to increase producer awareness of the importance of udder and teat hygiene in prevention of mastitis. Our results provide scientific evidence to support the commonsense notion that cows with a poor hygiene are at a greater risk of exposure to *Klebsiella* mastitis pathogens.

ACKNOWLEDGMENTS

This study was funded by the Northern New York Agricultural Development Program. We thank the Chilean Government, and Universidad de Concepción, Chile, for financial support of Marcos Munoz. The participation of dairy producers and QMPS personnel in northern New York in the field and laboratory components of this study is much appreciated.

REFERENCES

- Barkema, H. W., Y. H. Schukken, T. J. Lam, M. L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *J. Dairy Sci.* 81:411–419.
- Bartlett, P. C., G. Y. Miller, S. E. Lance, and L. E. Heider. 1992. Managerial determinants of intramammary coliform and environmental streptococci infections in Ohio dairy herds. *J. Dairy Sci.* 75:1241–1252.
- Bramley, A. J., and F. K. Neave. 1975. Studies on the control of coliform mastitis in dairy cows. *Br. Vet. J.* 131:160–169.
- Carroll, E. J., and D. E. Jasper. 1978. Distribution of Enterobacteriaceae in recycled manure bedding on California dairies. *J. Dairy Sci.* 61:1498–1508.
- Cook, N. B., and D. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Pages 31–43 in Proc. Natl. Mastitis Council. Annu. Mtg. Natl. Mastitis Council, Inc., Verona, WI.
- DeHart, D. A., R. P. Natzke, and P. A. Oltenacu. 1976. Effect of coliform challenge at milking time on new udder infections. *J. Dairy Sci.* 59:1124–1130.
- Dohoo, I., W. Martin, and H. Stryhn. 2003. Veterinary Epidemiologic Research. AVC Inc., Charlottetown, Prince Edward Island, Canada.
- Erschine, R. J., P. C. Bartlett, J. L. VanLente, and C. R. Phipps. 2002. Efficacy of systemic ceftiofur as a therapy for severe clinical mastitis in dairy cattle. *J. Dairy Sci.* 85:2571–2575.
- Galton, D. M., L. G. Peterson, and W. G. Merrill. 1988. Evaluation of udder preparations on intramammary infections. *J. Dairy Sci.* 71:1417–1421.
- Hogan, J., and K. L. Smith. 2003. Coliform mastitis. *Vet. Res.* 34:507–519.
- Hogan, J. S., K. L. Smith, K. H. Hoblet, D. A. Todhunter, P. S. Schoenberger, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, and B. L. Brockett. 1989. Bacterial counts in bedding materials used on nine commercial dairies. *J. Dairy Sci.* 72:250–258.
- Hogan, J. S., S. L. Wolf, and C. S. Petersson-Wolfe. 2007. Bacterial counts in organic materials used as free-stall bedding following treatment with a commercial conditioner. *J. Dairy Sci.* 90:1058–1062.
- Hughes, J. 2001. A system for assessing cow cleanliness. In *Practice* 23:517–524.
- Kikuchi, N., C. Kagota, T. Nomura, T. Hiramune, T. Takahashi, and R. Yanagawa. 1995. Plasmid profiles of *Klebsiella pneumoniae* isolated from bovine mastitis. *Vet. Microbiol.* 47:9–15.
- Magnusson, M., A. Christiansson, B. Svensson, and C. Kolstrup. 2006. Effect of different premilking manual teat-cleaning methods on bacterial spores in milk. *J. Dairy Sci.* 89:3866–3875.
- Munoz, M. A., C. Ahlstrom, B. J. Rauch, and R. N. Zadoks. 2006. Fecal shedding of *Klebsiella pneumoniae* by dairy cows. *J. Dairy Sci.* 89:3425–3430.
- Munoz, M. A., F. L. Welcome, Y. H. Schukken, and R. N. Zadoks. 2007. Molecular epidemiology of two *Klebsiella pneumoniae* mastitis outbreaks on a dairy farm in New York State. *J. Clin. Microbiol.* 45:3964–3971.
- Munoz, M. A., and R. N. Zadoks. 2007. Patterns of fecal shedding of *Klebsiella* by dairy cows. *J. Dairy Sci.* 90:1220–1224.
- National Mastitis Council. 1999. Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI.
- Paulin-Curlee, G. G., R. S. Singer, S. Sreevatsan, R. Isaacson, J. Reneau, D. Foster, and R. Bey. 2007. Genetic diversity of mastitis-associated *Klebsiella pneumoniae* in dairy cows. *J. Dairy Sci.* 90:3681–3689.
- Reneau, J. K., A. J. Seykora, B. J. Heins, M. I. Endres, R. J. Farnsworth, and R. F. Bey. 2005. Association between hygiene scores and somatic cell scores in dairy cattle. *J. Am. Vet. Med. Assoc.* 227:1297–1301.
- Roberson, J. R., L. D. Warnick, and G. Moore. 2004. Mild to moderate clinical mastitis: efficacy of intramammary amoxicillin, frequent milk-out, a combined intramammary amoxicillin, and frequent

- milk-out treatment versus no treatment. *J. Dairy Sci.* 87:583–592.
- Schreiner, D. A., and P. L. Ruegg. 2002. Effects of tail docking on milk quality and cow cleanliness. *J. Dairy Sci.* 85:2503–2511.
- Schreiner, D. A., and P. L. Ruegg. 2003. Relationship between udder and leg hygiene scores and subclinical mastitis. *J. Dairy Sci.* 86:3460–3465.
- Schukken, Y. H., G. van Schaik, J. J. McDermott, G. J. Rowlands, S. M. Nagda, W. Mulatu, and G. D. d'Ieteren. 2004. Transition models to assess risk factors for new and persistent trypanosome infections in cattle—analysis of longitudinal data from the Ghibe Valley, southwest Ethiopia. *J. Parasitol.* 90:1279–1287.
- Thomas, C. B., D. E. Jasper, M. H. Rollins, R. B. Bushnell, and E. J. Carroll. 1983. Enterobacteriaceae bedding populations, rainfall and mastitis on a California dairy. *Prev. Vet. Med.* 1:227–242.
- Todhunter, D. A., K. L. Smith, J. S. Hogan, and P. S. Schoenberger. 1991. Gram-negative bacterial infections of the mammary gland in cows. *Am. J. Vet. Res.* 52:184–188.
- Ward, W. R., J. W. Hughes, W. B. Faull, P. J. Cripps, J. P. Sutherland, and J. E. Sutherst. 2002. Observational study of temperature, moisture, pH and bacteria in straw bedding, and faecal consistency, cleanliness and mastitis in cows in four dairy herds. *Vet. Rec.* 151:199–206.
- Zadoks, R. N., M. A. Munoz, H. Griffiths, G. Bennett, and Y. H. Schukken. 2008. *Klebsiella*—Not by bedding alone. Pages 240–241 in Proc. Natl. Mastitis Counc. Annu. Meet. Natl. Mastitis Counc. Inc., Verona, WI.
- Zdanowicz, M., J. A. Shelford, C. B. Tucker, D. M. Weary, and M. A. von Keyserlingk. 2004. Bacterial populations on teat ends of dairy cows housed in free stalls and bedded with either sand or sawdust. *J. Dairy Sci.* 87:1694–1701.