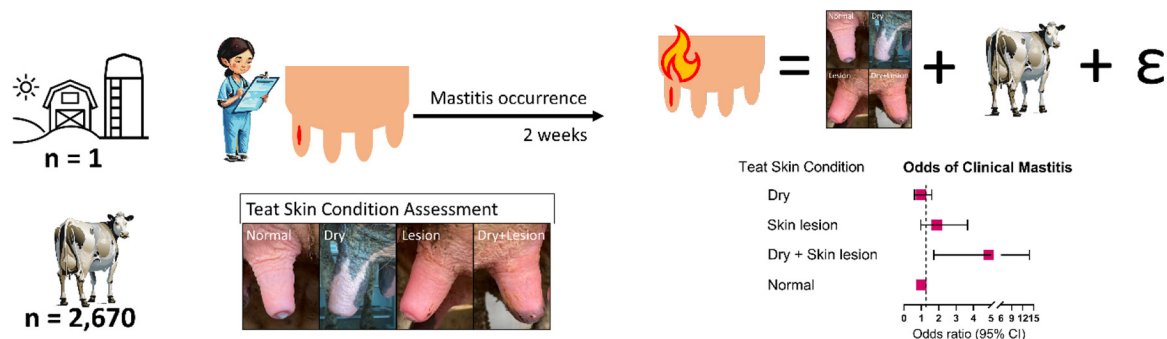


An observational study to investigate the association of teat skin condition with clinical mastitis risk

M. Wieland,^{1*} P. S. Basran,² P. D. Virkler,¹ and W. Heuwieser¹

Graphical Abstract

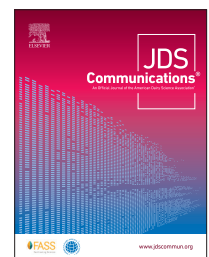


Summary

Teat skin condition from 2,670 dairy cows from a single dairy farm was evaluated and categorized as (1) normal, (2) dry skin, (3) skin lesion, and (4) dry skin and skin lesion. Cows were monitored for a total of 2 weeks after the assessment of the teat skin condition, and clinical mastitis occurrence at the quarter level was documented. A generalized linear mixed model was used to study the association between teat skin condition and clinical mastitis occurrence. Compared with quarters with teats with normal teat skin, the odds (95% confidence intervals) of clinical mastitis were 0.98 (0.60–1.60) for teats with dry skin, 1.88 (0.97–3.66) for teats with a skin lesion, and 4.87 (1.71–13.85) for teats with dry skin and a skin lesion.

Highlights

- We investigated the association between teat skin condition and clinical mastitis risk.
- Teat skin condition and occurrence of clinical mastitis at the quarter level were analyzed.
- We found an association between teat skin condition and the odds of clinical mastitis occurrence.
- Quarters with teats with dry skin and skin lesions had higher odds of clinical mastitis.



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An observational study to investigate the association of teat skin condition with clinical mastitis risk

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Abstract: The importance of teat canal integrity and its adjacent tissues in the dynamics of IMI is well documented, whereas research on the relationship between teat skin condition and clinical mastitis occurrence is scarce. The objective of this prospective cohort study was to investigate the association of teat skin condition with clinical mastitis occurrence in a closed cohort from a commercial dairy farm with a thrice daily milking schedule in the Northeast United States. We tested the hypothesis that quarters with teats with altered skin condition would have higher odds of clinical mastitis than those with normal skin. Teat skin condition from 2,670 cows was assessed during a single visit and categorized into (1) normal, (2) dry skin, (3) skin lesion, and (4) dry skin and skin lesion. Cows were monitored for 2 wk after the teat skin condition assessment, and the occurrence of clinical mastitis at the quarter level was documented. A generalized linear mixed model with a logit link and a binomial distribution revealed an association between teat skin condition and the occurrence of clinical mastitis. Compared with quarters with teats with normal teat skin, the odds (95% CI) of clinical mastitis were 0.98 (0.60–1.60) for teats with dry skin, 1.88 (0.97–3.66) for teats with a skin lesion, and 4.87 (1.71–13.85) for teats with dry skin and a skin lesion. We conclude that quarters from teats with dry skin and skin lesions had higher odds of clinical mastitis. In addition, we found evidence that quarters with teats with skin lesions have higher odds of clinical mastitis than those with normal teat skin, though future studies are needed. The results from this study show that teat skin condition should be considered in mastitis control programs on dairy operations.

Mastitis, the inflammation of the mammary gland, is one of the most important diseases in dairy cows with well-known adverse effects on animal welfare and the profitability of dairy operations (Ruegg, 2017). The bovine teat has long been recognized for its importance in the dynamics of IMI. It is also referred to as the first-line defense against IMI (Neijenhuis et al., 2001). The integrity of the tissue and skin of the teat is therefore critical to resisting IMI. Healthy teat skin is coated with a mantle of fatty acids with bacteriostatic properties that inhibit pathogen growth (Chikakane and Takahashi, 1995). The disruption of this protective mantle through, for example, mechanical forces or chemical irritants, may predispose the teat to colonization with mastitis pathogens leading to IMI. The teat skin is exposed to a plethora of mechanical, chemical, microbial, and ambient influences that impede its ability to maintain its integrity. Changes in teat skin integrity are reflected by skin dryness (i.e., scaly, flaky, or rough skin) and skin lesions (i.e., cracks, chaps, cuts, incisions, burns; Morton et al., 1987; Fox et al., 1991; Mein et al., 2001). These alterations of the teat skin integrity have been thought to increase teat skin colonization with *Staphylococcus aureus* (Fox et al., 1991; Fox and Cumming, 1996) and negatively affect udder health (Neave et al., 1969; Agger and Willeberg, 1986; Morton et al., 1987). However, over the last 3 decades, research investigating the relationship between teat skin condition and clinical mastitis has been scarce. Our objective therefore was to address this paucity and investigate the association of teat skin condition with clinical mastitis risk. We hypothesized that quarters from teats with altered skin condition (i.e., dry skin and

skin lesion) would have higher odds of clinical mastitis occurrence compared with quarters with normal teat skin.

This study was conducted at a 4,200-cow dairy located near Ithaca, New York. The farm was selected based on the owners' willingness to participate in the study. The study protocol was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (protocol no. 2020–0004). Cows were housed in freestall pens, bedded with manure solids, and fed a TMR. The herd was enrolled in monthly DHI(A) testing services, including the individual-cow SCC option. Herd data were recorded with a dairy management software program (Dairy Comp 305, Valley Agricultural Service). The key performance indicators were average daily milk production, 39.9 kg/d; 305-d mature equivalent milk production, 13,940 kg; mean test day SCC, 264,000 cells/mL; monthly clinical mastitis incidence, 14%; and pregnancy rate, 27%. Cows were milked 3 times per day in 8-h intervals with a 100-stall rotary parlor. The receiver operator vacuum was set to 46.1 kPa (13.6 inHg), leading to an average claw vacuum of 39 kPa (11.5 inHg). The pulsators were set to a rate of 60 cycles per minute and a ratio of 65:35. The cluster remover milk flow threshold was set at 1.3 kg/min with a 2-s delay time and a vacuum decay time of 1.4 s. The milk sweep was inactivated. The parlor was operated by two 12-h work shifts each consisting of 4 milking technicians. The premilking udder preparation consisted of (1) cleaning of teats with an automated teat brush that was rinsed with a chlorine-based (2,500 mg/kg) teat disinfectant at stall 1, (2) forestripping of 2 teats and application of a chlorine-based (2,500 mg/kg) teat disinfectant

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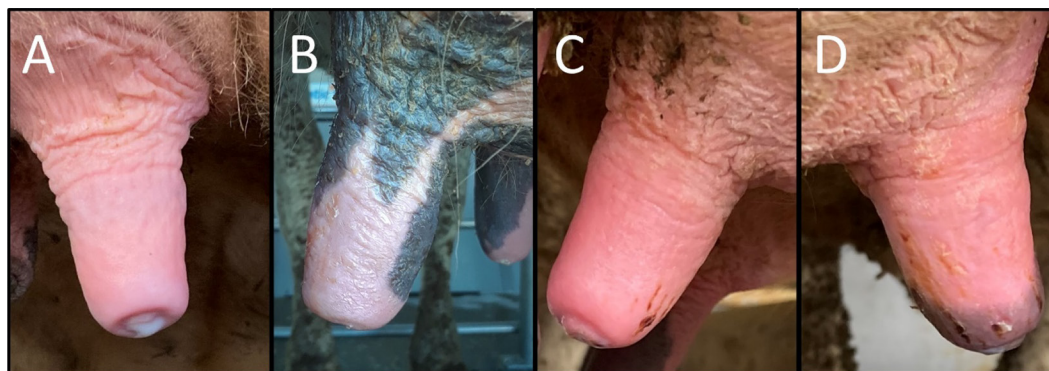


Figure 1. Teat skin condition categories: (A) normal (smooth), (B) dry skin, (C) skin lesion present, and (D) dry skin and skin lesion present.

to all teats at stall 3, (3) wiping of teats with a cloth towel at stall 14, and (4) attachment and alignment of the milking unit at stall 20 for early- and mid-lactation cows and stall 25 for late-lactation cows. To ensure consistent monitoring of quarters for the presence of clinical mastitis, the teats were forestripped in an alternating fashion, such that the side (i.e., left side, left hind and left front; or right side; right hind and right front) was changed for each milking session. The rotational speed was 4.9 s/stall and led to a dip contact time of 54 s, a stimulation duration of 9 s, and a preparation lag time of 93 s for early- and mid-lactation cows and 118 s for late-lactation animals. The iodine-based postmilking teat disinfectant was applied with 2 teat spray robots at stalls 90 to 93.

This prospective cohort study lasted from February 22 to March 8, 2022. We chose to monitor the occurrence of clinical mastitis cases for a total duration of 2 wk after the assessment. Our reasoning was based on our experience that meaningful changes in dry teat skin condition occur over a period of 2 wk. We therefore hypothesized that the teat skin condition assessed during a single assessment reflected the condition and the teat's susceptibility to clinical mastitis occurrence in a 2-wk period after the assessment. All cows with a calving date of February 22, 2022, or earlier that were in the lactating herd on the day of the assessment were eligible for enrollment. To avoid a potential selection bias, cows that were in the hospital pen on the day of assessment were not included. We conducted no a priori sample size calculation but elected to enroll as many cows as could be accomplished during the assessment of the teat skin condition. The study population, therefore, consisted of a convenience sample of all lactating cows with a calving date of February 22, 2022, or earlier, for which teat skin condition data were obtained.

The teat skin condition of all lactating quarters was evaluated by a trained investigator (MW) during a single morning milking session according to the guidelines provided by the National Mastitis Council (NMC, 2007). Briefly, after completion of the premilking udder preparation but before machine milking, the teat skin condition was evaluated through manual palpation of the teat skin and the visual inspection of the entire teat barrel and the teat orifice. Manual palpation was conducted with latex-gloved hands by dragging a finger and thumb along the teat barrel. The presence of skin lesions (e.g., chaps, abrasions, cuts, and frostbite) was documented by means of visual assessment. The teat skin condition along with the quarter position was then recorded and classified into (1) nor-

mal (smooth), (2) dry skin (slightly rough [with some drag] or very rough [when the latex puckers]), (3) skin lesion present, or (4) dry skin and skin lesion present. Figure 1 illustrates the 4 teat skin condition categories. The presence and position of nonlactating quarters were also documented. The teat skin condition of nonlactating quarters was not evaluated for apparent reasons.

Clinical mastitis detection was performed by trained milking technicians during the premilking udder preparation and was based on the milk character and signs of inflammation (i.e., heat, pain, redness, and swelling) of the affected mammary gland. Clinical mastitis was defined as present if milk from 1 or more quarters was abnormal. The farm management employed pathogen-based mastitis treatment. Thus, an aseptic milk sample was taken from mastitic quarters at the time of detection and submitted to the Animal Health Diagnostic Center (Cornell University) for bacteriological testing. Data on lactation number, stage of lactation, the SCC from the last DHI(A) test day, linear somatic cell score (LS), the occurrence of clinical mastitis events, and the respective culture results during the study period were extracted from the farm management software program (Dairy Comp 305, Valley Agricultural Software).

Data were compiled in Excel (Microsoft Corp.) and JMP Pro (version 15, SAS Institute Inc.). Data from cows that left the lactating herd before March 8, 2022, were included up until the time of removal. Before the analyses, we screened the data for missing and erroneous values. Statistical analyses were performed with SAS (version 9.4, SAS Institute Inc.). We fitted a generalized linear mixed model with a logit link and a binomial distribution to study the association between teat skin condition and clinical mastitis occurrence. The cow was included as a random effect to account for the dependence of quarters within the cow. The covariance was modeled with the variance component covariance structure. The dependent variable was the occurrence (present vs. absent) of the first clinical mastitis event at the quarter level during the study period. The teat skin condition (normal, dry skin, skin lesion, and dry skin and skin lesion) was the independent variable of interest and included as a fixed effect. Lactation number (first, second, and third or greater), stage of lactation (≤ 100 , 101–200, and >200 DIM), the cow's last test day LS, quarter position (front vs. hind quarters), and the presence (or absence) of a nonlactating quarter were considered covariates and screened for inclusion in the initial model through univariable analyses. Covariates that led to a P -value < 0.20 were deemed eligible for inclusion. Spearman cor-

Table 1. Frequency distribution of culture results from milk samples collected from 136 clinical mastitis cases

Culture result	Number	Percentage
<i>Streptococcus uberis</i>	40	29.4
Negative	39	28.7
<i>Streptococcus</i> spp.	10	7.4
<i>Staphylococcus aureus</i>	9	6.6
<i>Escherichia coli</i>	8	5.9
<i>Klebsiella</i> spp.	7	5.1
<i>Streptococcus dysgalactiae</i>	7	5.1
<i>Staphylococcus</i> spp.	3	2.2
Contamination	2	1.5
No culture results available	2	1.5
<i>Pasteurella</i> spp.	2	1.5
<i>Staphylococcus aureus</i> and <i>Streptococcus uberis</i>	2	1.5
<i>Mycoplasma</i> spp.	1	0.7
Other	1	0.7
<i>Staphylococcus aureus</i> and <i>Streptococcus dysgalactiae</i>	1	0.7
<i>Streptococcus uberis</i> and <i>Klebsiella</i> spp.	1	0.7
<i>Streptococcus uberis</i> and other	1	0.7

relation coefficients were calculated to test for collinearity among eligible covariates and a coefficient of $|\geq 0.55|$ was regarded as the threshold value. To reach the final model, manual backward selection was performed until all variables had a P -value < 0.05 . Two-way interactions between teat skin condition and the remaining covariates were tested one at a time and retained in the model if P -value < 0.05 . Finally, the adjusted probabilities and 95% CI were calculated. Last, we conducted post hoc power analyses in JMP Pro (version 15, SAS Institute Inc.) using the observed frequency distribution for mastitis occurrence and teat skin condition category.

We obtained teat skin condition data from 2,670 cows. Exclusion of teat observations from nonlactating quarters and those with missing teat skin condition scores resulted in a total of 10,158 quarter observations. Two recurrent mastitis cases were excluded from the analyses (data for the first case were retained). Cows were in their first ($n = 922$, 34.5%), second ($n = 757$, 28.4%), and third or greater ($n = 991$, 37.1%) lactation and between 2 and 670 DIM (mean \pm SD, 138 ± 92 d). The median SCC and mean (\pm SD) LS were 42,000 cells/mL and 2.2 ± 1.9 , respectively. A nonlactating quarter was documented in 452 (16.9%) cows. Teat skin condition was distributed as follows: normal, 8,066 (79.4%); left front quarter [LF], 2,099; left hind quarter [LH], 1,978; right front quarter [RF], 2,025; right hind quarter [RH], 1,964; dry skin, 1,634 (16.1%); LF, 398; LH, 363; RF, 471; RH, 402; skin lesion, 394 (3.9%); LF, 29; LH, 179; RF, 21; RH, 165; and dry skin and skin lesion, 64 (0.6%); LF, 8; LH, 23; RF, 5; RH, 28). A total of 136/10,158 (1.3%) clinical mastitis cases were documented. A total of 36/2,534 (1.4%) cases were detected in the LF, 38/2,534 (1.5%) in the LH, 31/2,522 (1.2%) in the RF, and 31/2,559 (1.2%) in the RH quarter. The frequency distribution of mastitis cases among teats with different skin condition was as follows: normal, 99/8,066 (1.2%); dry skin, 22/1,634 (1.4%); skin lesion, 11/394 (2.8%); and dry skin and skin lesion, 4/64 (6.3%). Table 1 shows the results from bacteriological testing of mastitic quarters.

Based on univariable analyses, all covariates but quarter position ($P = 0.9$) were eligible for inclusion in the initial model ($P \leq 0.0001$). Spearman correlation coefficients revealed no collinearity among eligible covariates ($r \leq |0.12|$); thus, all eligible covariates were included in the initial model. All covariates remained in the model, but none of the tested 2-way interactions were retained.

The final multivariable model included lactation number ($P = 0.01$), stage of lactation ($P < 0.0001$), presence of a nonlactating quarter ($P < 0.0001$), LS ($P < 0.0001$), and teat skin condition ($P = 0.008$). Figure 2 shows the final model's odds ratios, 95% CI, and P -values. The adjusted probabilities (95% CI) for the occurrence of a clinical mastitis event were 1.2% (0.9%–1.5%) for normal teat skin, 1.1% (0.7%–1.8%) for dry teat skin, 2.2% (2.0%–4.1%) for skin lesion, and 5.4% (2.0%–13.9%) for dry skin and skin lesion.

Using the frequency distributions of clinical mastitis occurrence for each teat skin condition category (normal, 1.2%; dry skin, 1.4%; skin lesion, 2.8%; and dry skin and skin lesion, 6.3%), the number of teats in each category (normal, 8,066; dry skin, 1,634; skin lesion, 394; and dry skin and skin lesion, 64), an α -level of 0.05, a 2-sided test, and normal teat skin as the baseline value for comparison, yielded power values of 0.09 for dry teat skin, 0.56 for skin lesion, and 0.46 for dry skin and skin lesion, respectively.

In this study, we investigated the association of teat skin condition with the occurrence of clinical mastitis in a closed cohort from a single New York dairy farm. Our data support our hypothesis and indicate that quarters with teats with dry skin and skin lesions had higher odds of clinical mastitis than those with normal teat skin. In addition, we found evidence that quarters with teats with skin lesions may have higher odds of clinical mastitis than those with normal teat skin. Several authors have described a relationship of poor teat skin condition with IMI (Neave et al., 1969; Fox et al., 1991) and mastitis (Francis, 1984; Morton et al., 1987). Our data add to the existing body of literature and suggest that teat skin condition is a risk factor for clinical mastitis.

We believe that the higher odds of clinical mastitis occurrence in quarters with teats with dry skin and skin lesions have been caused by an increased teat skin colonization with mastitis-causing pathogens, resulting in a greater risk of teat orifice colonization, new IMI, and clinical mastitis. This is supported by results from previous studies showing that poor teat skin condition was positively correlated with *Staphylococcus aureus* colonization (Fox et al., 1991; Fox and Cumming, 1996), and that an enhanced teat skin colonization with *Staphylococcus aureus* was associated with greater teat orifice colonization with *Staphylococcus aureus* and IMI (Fox et al., 1991).

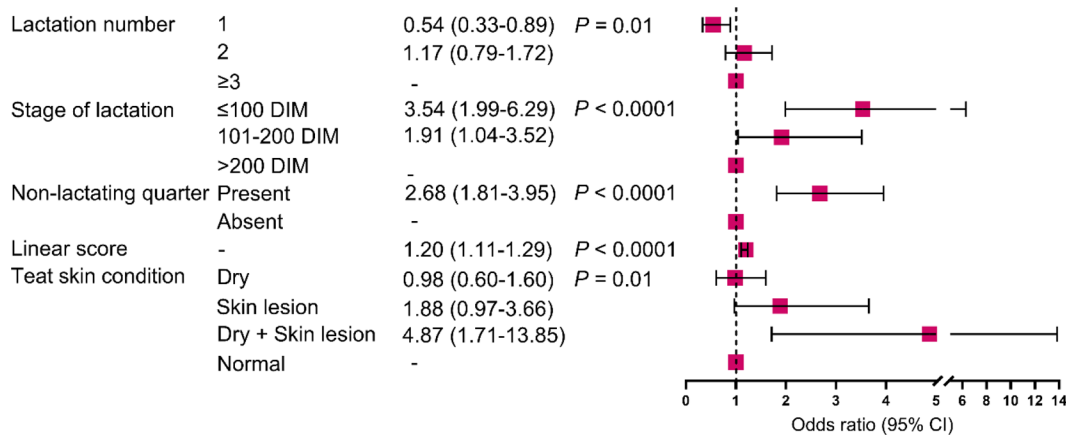


Figure 2. Results from multivariable generalized linear mixed model showing the association of lactation number, stage of lactation, presence of a nonlactating quarter, linear SCS, and teat skin condition with the occurrence of clinical mastitis. Adjusted odds ratios, 95% CI, and P -values are presented.

We also speculate that teats with dry skin and skin lesions hampered the milking technicians' ability to sanitize the teats before milking, as reported by Bushnell (1985), thereby leading to a higher pathogen load at the teat skin and orifice, which has been considered a risk factor for IMI (Pankey et al., 1984). Our theory is supported by findings from Neave et al. (1969) who investigated the effect of milking hygiene measures (i.e., use of disinfectants, paper towels or boiled cloths, wearing rubber gloves, pasteurization of the teat cups, and the application of postmilking teat disinfectant) on IMI. The researchers documented that milking hygiene measures reduced the proportion of *Staphylococcus aureus*-colonized teats but found that measures were less effective in teats with chaps and sores (Neave et al., 1969). We also believe that this theory may apply to other pathogens that have been found in mastitic milk samples from the current study and isolated from dairy cows' teat skin in previous ones (Baumberger et al., 2016; Guarín et al., 2017). Last, it is also possible that teat skin lesions reflect compromised defense mechanisms, including a decreased ability to maintain the protective acid mantle, which has been thought to predispose the teat to colonization with pathogens (Fox et al., 2003).

The absence of differences in the odds of clinical mastitis for quarters with teats with dry skin only supports descriptions by other authors (Mein et al., 2001) and could be due to the dairy farm's premilking udder preparation regimen consisting of teat brushing with an automated teat brush, 2 applications of teat disinfectants before milking, and a wiping step with an individual cloth towel. It is therefore possible that clinical mastitis occurrence in quarters with teats with dry skin would have been higher on dairy herds with a less stringent premilking teat sanitization regimen.

Risk factors for dry teat skin condition and teat skin lesions include exposure to cold and windy conditions (Mein et al., 2001), extreme temperatures (Burmeister et al., 1995), faulty milking machine settings (Jackson, 1970), bedding characteristics (e.g., moisture) and bedding additives such as quick lime (calcium oxide) or hydrated lime (calcium hydroxide) (Gleeson, 2013; Virkler and Wieland, 2023), and postmilking teat disinfectants (e.g., chlorine-based disinfectants) (Burmeister et al., 1998). The prevention of poor teat skin condition therefore includes protecting cows' teats

from harsh weather conditions (e.g., installation of draft shields), machine milking equipment maintenance, and mitigating the negative impact of bedding additives or teat disinfectants (e.g., addition of emollient to the postmilking teat disinfectant). The implementation of such control measures can be cost-intensive. We believe that data generated from studies like the one presented here can guide management decisions on dairy operations and inform a partial budget to compare the costs and benefits of proposed management changes or the implementation of control measures.

We found lower odds of clinical mastitis in first-lactation animals compared with those in lactation 3 or greater. This finding is in accordance with that from previous work (Zadoks et al., 2001) and, as previously discussed (Lean et al., 2023), is thought to be due to changes in the anatomy of the mammary gland and teats with progressing lactation number, as well as parity-associated metabolic changes such as the disruption of the calcium homeostasis, which in turn has been associated with increased mastitis risk (Curtis et al., 1983). The higher odds of clinical mastitis in early- (≤ 100 DIM) and mid-lactation (101–200 DIM) animals as compared with cows that were >200 DIM also supports descriptions by other researchers (Barkema et al., 1998). As discussed by Oliver and Sordillo (1988), the physiological transition of the mammary gland to a state of active milk synthesis after parturition could be associated with increased susceptibility to new IMI and explain the observed phenomenon. In accordance with previous work (Wieland and Skarbye, 2024), cows with a nonlactating quarter had higher odds of clinical mastitis. Last, a 1-unit increase in LS increased the odds of clinical mastitis by 20%, a finding that supports the results reported by other investigators (Steenefeld et al., 2008).

Our study had some limitations that the reader should consider. First, we conducted this study on a single commercial dairy farm in New York State. The external validity of our results is therefore limited to similar dairy operations in this region. Future studies should enroll cows from different regions and dairy operations with different management systems to facilitate generalizability. Second, the enrollment of a convenience sample of cows could have led to selection bias. Third, teat skin condition was assessed during a single visit by means of manual palpation and visual inspection by one investigator. This subjective assessment was likely

subject to operator fatigue and information bias. Fourth, this study was limited to the assessment of teat skin condition, whereas data on teat tissue condition (e.g., teat-end condition) have not been considered. Future studies should therefore strive to use automated scoring systems that facilitate the serial assessment of teat skin condition over time through, for example, machine learning approaches and include additional teat tissue condition traits such as teat-end condition. Similarly, due to only 2 teats being forestripped at a given time, the ability to detect clinical mastitis cases might have been limited. Last, due to the observational nature of this study, a cause-effect relationship cannot be inferred.

In the study cohort presented here, quarters from teats with dry skin and skin lesions had higher odds of clinical mastitis. In accordance with previous reports, we attributed these findings to increased teat skin colonization with mastitis pathogens, interference of teat lesions with teat sanitization, and decreased defense mechanisms of teats with skin lesions. The results from our study highlight the importance of teat skin condition as a risk factor for clinical mastitis. Future studies with a larger sample size considering different regions and management strategies are warranted.

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Nonstandard abbreviations used: CIDA = Cornell Institute for Digital Agriculture; LF = left front quarter; LH = left hind quarter; LS = linear somatic cell score; RF = right front quarter; RH = right hind quarter.