J. Dairy Sci. 100:643–652
https://doi.org/10.3168/jds.2016-11459

Association of anatomical characteristics of teats with quarter-level somatic cell count

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ABSTRACT

Mastitis occurs after bacteria successfully traverse the teat orifice and cause an intramammary infection. Anatomical characteristics of the teat are potential risk factors for infection. The objective of this study was to identify potential associations between anatomical characteristics of teats and quarter-level somatic cell count (QSCC) from cows on larger dairy farms in Wisconsin. Teat dimensions (length and diameter at the teat barrel and apex) were measured, and hyperkeratosis scores were assessed for 3,713 quarters of 959 cows on 9 dairy farms. The SCC of quarter milk samples obtained from those teats was determined. Multivariate models were used to determine associations of teat anatomical characteristics with QSCC. Subclinical mastitis was defined as a quarter milk sample with SCC of >150,000 cells/mL. Teat dimensions and milk components varied among farms. In the group of farms enrolled in this study, prevalence of subclinical mastitis in mammary gland quarters ranged from 13.6 to 28.9%. An interaction of teat apex diameter and quarter position (front or rear) was identified for QSCC. For both front and rear quarters, a tendency existed for narrower teat barrels to be associated with increased QSCC. However, for front quarters only, greater diameter of the teat apex was associated with increased QSCC. Teat shape (square or triangular teats) was not associated with QSCC. Milk samples obtained from teats with hyperkeratosis scores of very rough had greater QSCC compared with milk samples obtained from teats with hyperkeratosis scores of normal, smooth ring, or rough ring.

Key words: dairy, subclinical mastitis, milking machine, teat

INTRODUCTION

Mastitis is defined as inflammation of the mammary gland in response to entry of pathogenic microorganisms through the teat canal, resulting in an IMI (Hogan et al., 1999). Mastitis causes losses in milk production (Hortet and Seegers, 1998) and reduces the processing capabilities of milk obtained from affected glands. Its harmful effects include the temporary or permanent loss of milk production, reduced milk quality, reduced milk value, need to discard of milk after antibiotic treatment, increased treatment and labor costs, increased costs for disease surveillance, and premature culling or reduced productive life of cows (Hortet and Seegers, 1998; Klei et al., 1998; Viguier et al., 2009).

The teat canal is a natural barrier against mastitis pathogens (Sordillo et al., 1997; Paulrud, 2005). In recent years, dairy cattle have been selected for increased milking speed and parlor throughput (Ruegg and Erskine, 2014). Additionally, modern farms tend to increase milking vacuum, which has led to reduced efficacy of teat canal defense mechanisms and potentially increased risk of IMI (Moore et al., 1983; Reinemann et al., 2008; Neijenhuis, 2011). Wider teat canals and sphincter muscles that cannot efficiently close the teat orifice have both been associated with wider teat diameters, increased milk flow rates, and greater milk production (Seykora and McDaniel, 1985). Teats that have a dysfunctional sphincter that fails to effectively close the teat orifice are more likely to develop IMI compared with teats with functional sphincters (Seykora and McDaniel, 1985). Milking machine–induced changes in teat-end condition may influence the occurrence of IMI by increasing teat congestion, altering keratin dynamics, and altering the closure of the teat orifice after milking and by increasing the proportion of teats with greater hyperkeratosis scores (Mein et al., 2004).

In a case–control study performed using a single herd (Guarín and Ruegg, 2016), we demonstrated that twice as many clinical mastitis cases occurred in front quarters compared with rear quarters, possibly because of anatomical differences between front and rear teats. Compared with rear teats, front teats were larger and wider at the teat barrel and tip (Guarín and Ruegg, 2016). Increased premilking diameter of the teat apex was associated with increased risk of clinical mastitis, but milking machine–related changes in teat dimen-

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Received May 13, 2016.
Accepted September 14, 2016.
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sions were not associated with risk of clinical mastitis. Associations among teat dimensions, teat shape, and mastitis have not been clearly established, especially for management used on modern large dairy farms.

The objective of the study was to examine anatomical characteristics of teats (including teat shape, the length and diameter at the teat barrel and teat apex, and occurrence of teat-end hyperkeratosis) and determine associations between these characteristics and prevalence of subclinical mastitis in dairy cows on modern farms in Wisconsin.

MATERIALS AND METHODS

The study was conducted on commercial dairy farms (n = 9) in Wisconsin between June and September 2015. All procedures used in this study were approved by the Animal Care and Use Committee at the University of Wisconsin-Madison (protocol no. A-01-553).

Study Design

The study was a cross-sectional study to determine associations between teat anatomical characteristics (including hyperkeratosis) and subclinical mastitis. The null hypothesis was that no association existed between teat anatomical characteristics and quarter-level SCC (**QSCC**). The experimental unit was quarter within cow.

A Priori Sample Size Determination

Sample size was calculated to achieve a 95% confidence and 80% power to detect differences of at least 35,000 cells/mL in the QSCC analysis. The variability of SCC by parity group (1, 2, and 3+) within a population of more than 400 Holstein cows (Guarín, 2016) was used to calculate the minimum sample size to detect variations at different ranges of SCC. Detection of the desired QSCC difference required a minimum of 2,892 quarters. An additional 20% of quarters were added to allow for sampling within cows and expected data loss, resulting in a total estimated sample size of 3,470 quarters. Sampling was distributed among parity groups with about 33% collected from each parity group 1, 2, and ≥3). Based on the assumption that each cow has 4 teats, about 80 to 100 cows were enrolled from each of the 9 farms that participated in the study.

Inclusion Criteria for Farms, Cows, and Quarters

To be eligible for inclusion in the study, farms were required to have at least 500 cows, participate in monthly DHIA testing, use Dairy Comp 305 (Valley Agricultural Software, Tulare, CA), and agree to participate in the study by signing an informed consent document. All Holstein milking cows with at least 3 functional quarters were potentially eligible for the study. Quarters were eligible for inclusion if they were functional, had no visible signs of clinical mastitis when forestripped, and were producing marketable milk on the day of sampling.

Definition of Subclinical Mastitis

Quarter milk samples collected from enrolled quarters were preserved with bronopol and submitted for SCC analysis by flow cytometry at a commercial DHIA laboratory (AgSource Cooperative Resources International, Verona, WI). Subclinical mastitis was defined as quarter milk with SCC >150,000 cells/mL (Fuenzalida et al., 2015). Milk samples were also analyzed for butterfat, protein, lactose, and solids-not-fat.

Sample Collection and Randomization

University researchers visited each farm once during milking to collect milk samples and to measure and assess teats. Researchers arrived at the beginning of milking and enrolled cows as they entered the milking parlor, remaining until the end of the milking shift. Cows were systematically enrolled when they entered the milking parlor based on entry position. Only cows in the first 2 stalls on each side of the parlor were eligible to be enrolled in the study (n = 4 cows enrolled per parlor turn during the entire milking session on each farm). On all farms, the same researcher measured teat characteristics and assigned hyperkeratosis scores (JFG), while a second researcher (MGP) collected quarter milk samples used for determination of SCC. Teats were measured after premilking teat preparation was performed by a farm employee but before attachment of the milking unit. Teat dimensions were measured using a translucent measuring ruler with a scale unit of 2 mm, which illuminated the teats with a white lamp (WestfaliaSurge Inc., Naperville, IL). All measurements were video recorded to verify the information. A description of the teat segment measurements has been described previously (Guarín and Ruegg, 2016). Briefly, teat measurements included teat length (measured from the base to the teat end), teat diameter at the barrel (measured at the middle part of the teat), and teat diameter at the apex (approximately 10–15 mm proximal to the teat end). The distance proximal to the teat end was standardized as much as possible in the field by having a single experienced person record all measurements and by using the measurement marks of the ruler as a visual guide during data collection. Quarter milk samples were collected after foremilk was removed and milk was visually examined for abnormalities indicative of clinical mastitis. To ensure a representative sampling of cows within each herd, milk samples and teat measurements were distributed among all groups of clinically healthy cows on each farm.

Acquisition of Farm Management Data

Individual cow data were collected from herd management software and included parity, SCC, DIM, milk yield, and adjusted 305-d milk production. All data were entered into a database and checked for completeness and outliers.

Primary and Secondary Outcomes

Primary outcomes were QSCC, the number of quarters with >150,000 cells/mL, and cow-level SCC (from most recent DHIA test). The secondary outcome was hyperkeratosis score, which was defined using a 4-point ordinal scale (no ring, smooth or slight ring, rough ring, and very rough ring; Neijenhuis et al., 2001a). The QSCC and individual cow SCC data were $\log_{10}$ transformed to normalize their distributions.

Statistical Analysis

To determine if teat dimensions and milk components varied among farms, mixed models were used (PROC MIXED; SAS Institute Inc., 2013). The response variables of these models were the measured teat dimensions (teat length, teat diameter at the barrel, and teat diameter at the apex) and the quarter milk analysis results for butterfat, protein, lactose, and solids-nonfat. For the comparison among farms, position of the quarter (front or rear), parity group (1, 2, 3+), and farm were included as fixed effects in each model. Cow was used as compound symmetry term to account for lack of independence among quarters within each cow.

The model was

$$\text{Variable}\begin{bmatrix}\text{teat length,}\\ \text{teat diameter at the barrel,}\\ \text{teat diameter at the apex,}\\ \text{butterfat,}\\ \text{protein,}\\ \text{lactose,}\\ \text{solids-non-fat}\end{bmatrix}$$
$$= \mu + QuarterPosition_{ij} + Parity_j + Farm_k + Cow_{ij} + \varepsilon_{ijk},$$

where variable of interest = each response variable (teat length, teat diameter at the barrel, teat diameter at the apex, butterfat, protein, lactose, or solids-non-fat) of the *i*th quarter, the *j*th cow, and the *k*th herd; μ = regression intercept; $QuarterPosition_{ij}$ = quarter position (front or rear); $Parity_j$ = parity (1, 2, or ≥ 3); $Farm_k$ = fixed effect of the farm; Cow_{ij} = compound symmetry to account for the correlation among quarters within cow; and ε_{ijk} = residual variation.

The PROC TTEST (SAS Institute Inc., 2013) was used to perform paired-samples *t*-tests between front and rear teats in the same cow for each of the measured segments of the teats.

To test the null hypothesis that QSCC and teat dimensions (teat length, teat barrel diameter, and teat apex diameter) were not associated, 3 separate generalized linear mixed models were used. Each model was corrected for within-cow and within-farm clustering, using farm and cow within farm as random effects. Cow was used as a repeated measurement (compound symmetry) to account for the possible correlation among quarters within cow. All models were run using the PROC MIXED and included parity and DIM as covariables. The experimental unit was the quarter, and the main response variable was QSCC. Model [1] tested the hypothesis that QSCC was independent of teat dimensions:

$$\begin{aligned}\text{Log}_{10}QSCC_{ijk} &= \mu + Length_{ij} + Barrel_{ij} + Apex_{ij}\\ &+ QuarterPosition_{ij} + Apex \times QuarterPosition_{ij}\\ &+ Parity_j + DIM_j + Farm_k + Quarter(Cow)_{ij}\\ &+ Cow(Farm)_{jk} + \varepsilon_{ijk}, \qquad [1]\end{aligned}$$

where $\log_{10}QSCC_{ijk}$ = log base 10 of the QSCC from the *i*th quarter, *j*th cow, and *k*th herd; μ = regression intercept; $Length_{ij}$ = length of the teat (mm); $Barrel_{ij}$ = diameter of the barrel (mm); $Apex_{ij}$ = diameter of the teat apex (mm); $Apex \times QuarterPosition_{ij}$ = interaction of teat apex and quarter position; DIM_j = days in milk (d); $Quarter(Cow)_{ij}$ = compound symmetry to account for the correlation among quarters within cow; $Cow(Farm)_{jk}$ = random effect reflecting cow within herd; and other terms were as defined previously.

Model [1] demonstrated that teat position (front or rear) was associated with QSCC, so 2 separate models (model 2 for the front quarters and model 3 for the rear quarters) were subsequently used to determined association between teat dimensions and QSCC.

Models [2] (for front quarters) and [3] (for rear quarters) were

$$\text{Log}_{10}QSCC_{ijk} = \mu + Length_{ij} + Barrel_{ij} + Apex_{ij} + Parity_j + DIM_j + Farm_k + Quarter(Cow)_{ij} + Cow(Farm)_{jk} + \varepsilon_{ijk}, \qquad [2, 3]$$

where $\log_{10}QSCC_{ijk}$ [for front quarters (model [2]) or for rear quarters (model [3])] = log base 10 of the QSCC from the ith quarter, the jth cow, and the kth herd; and other terms were as defined previously.

Model [4] was used to test the hypothesis that QSCC and teat shape (square or triangular) were not associated. Teat shape was defined based on the relationship of teat barrel diameter and diameter at the teat apex. Teats that had a greater ratio of the barrel to apex dimensions than the third quartile of the distribution for this variable were categorized as squared, and those that had a lesser ratio than the third quartile were categorized as triangular teats. Model [4] was

$$\text{Log}_{10}QSCC_{ijk} = \mu + Shape_{ij} + Farm_k + Quarter(Cow)_{ij} + Cow(Farm)_{jk} + \varepsilon_{ijk}, \qquad [4]$$

where $Shape_{ij}$ = teat shape (square or triangular), and other terms were as defined previously.

Model [5] was used to test the hypothesis that no association existed between hyperkeratosis scores and QSCC. The response variable was QSCC. The explanatory variables were cow, farm, DIM, parity, and quarter location. Likewise, the same covariance structures of previous models were used in model [5]:

$$\text{Log}_{10}QSCC_{ijk} = \mu + Hyperkeratosis_{ij} + Parity_j + DIM_j + Farm_k + Quarter(Cow)_{ij} + Cow(Farm)_{jk} + \varepsilon_{ijk}, \qquad [5]$$

where $Hyperkeratosis_{ij}$ = hyperkeratosis score: no ring (**N**), smooth or slight ring (**S**), rough ring (**R**), to very rough ring (**VR**), and other terms were as defined previously.

All analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). Significance was declared at $P < 0.05$.

RESULTS

Descriptive Results

The experiment was conducted on 9 large Wisconsin dairies (average number of lactating cows was 1,424 ± 624; Table 1), all of which were milked in linear milking parlors. Teats that fit the enrollment criteria (n = 3,713; Table 1) were measured, evaluated for hyperkeratosis, and had quarter milk samples collected. Enrolled cows (n = 959) were 179 ± 125 DIM and had an adjusted 305-d milk production of 14,065 ± 2,596 kg. The median SCC of enrolled cows at the DHIA test the month before the farm visit was 37,500 cells/mL (range 4,000–9,701,000 cells/mL). Least squares means values for teat length varied among farms, ranging from 39.0 to 45.7 mm. The mean diameter of the teat barrel varied from 21.5 to 23.0 mm, and the diameter of the teat apex varied from 17.8 to 19.4 mm ($P < 0.05$).

Milk components were tested on samples collected after removal of foremilk but before milking. Among farms, LSM ranged from 1.7 to 2.7% for fat, 2.8 to 3.2% for protein, 4.6 to 4.9% for lactose, and 8.4 to 8.9% for solids-non-fat ($P < 0.05$). Based on a threshold of 150,000 cells/mL, quarter-level prevalence of subclinical mastitis ranged from 13.6 to 28.9% of quarters ($P < 0.05$).

Table 1. Characteristics of farms and cows enrolled in the study

All cows			Enrolled cows						
			Milk production (kg/d)		DHIA SCC $\times 10^3$ cells/mL				
Farm	Herd size[1]	Liner type	Mean	SD	Median	Range	Cows enrolled	Quarters enrolled	No. of nonfunctional quarters
A	1,164	Westfalia	39.4	9.9	33.0	6–2,501	117	457	11
B	2,638	Boumatic	38.4	9.4	36.0	7–2,413	99	376	20
C	2,334	Boumatic	37.4	10.2	36.0	9–3,119	119	463	13
D	1,637	Westfalia	40.2	6.6	32.0	5–8,571	129	493	23
E	691	Boumatic	48.1	12.1	31.0	13–2,263	115	430	30
F	1,334	Boumatic	45.7	9.3	27.0	5–1,595	90	359	1
G	939	Afimilk	49.1	12.8	21.5	4–3,224	100	394	6
H	946	Boumatic	36.3	10.1	70.0	13–9,701	88	338	14
I	1,135	Westfalia	42.5	9.4	51.0	6–3,697	102	403	5
Total	12,818	—	41.9[2]	10.9	37.5[2]	4–9,701	959	3,713	123

[1] Number of lactating cows.

[2] Average value for the enrolled cows.

Compared with rear teats, front teats were longer ($P < 0.001$) and wider at the teat apex ($P < 0.001$) but not at the teat barrel ($P = 0.901$; Table 2).

Teat Dimensions and QSCC

In model [1] (complete results of the model are not shown), DIM and parity group were positively associated with increased QSCC ($P < 0.001$). Teat position was associated with QSCC ($\beta = -0.312$ for front compared with rear teats; $P = 0.036$). Model [1] included the interaction term apex diameter × quarter position. Second- and third-level interactions were tested, but only the interaction of apex diameter × quarter position was significant ($P = 0.042$). Based on the interaction of apex diameter by position, separate models (models [2] and [3]; Table 3) were subsequently used to describe the associations of QSCC with teat dimensions. In both models, increased DIM and parity group were associated with increased QSCC ($P < 0.001$; Table 3). Teat length was not associated with QSCC, but as teat barrel diameter increased, a tendency was apparent for reduced QSCC for both front and rear quarters (Table 3). For front quarters only, greater diameter of the teat apex was associated with increased QSCC ($P = 0.005$; Table 3). Model [4] evaluated the association between teat shape and QSCC. As defined in this study, teat shape was not related to QSCC ($P = 0.\ 450$).

Of teats scored for hyperkeratosis (n = 3,693), 2,782 (75.3%) were scored N, 724 (19.6%) were scored S, 158 (4.3%) were scored R, and 29 (0.8%) were scored VR. After adjusting for the effects of parity and DIM, hyperkeratosis scores were associated with increased QSCC ($P = 0.\ 001$; Table 4). Compared with teats with scores of N, teats scored VR had greater QSCC ($P = 0.003$). In contrast, teats with scores of S had slightly decreased QSCC ($P = 0.039$).

DISCUSSION

During the period that this study was conducted, approximately 1,267,000 cows (USDA-NASS, 2014) were located on 10,290 Wisconsin dairy farms. Although the average herd size of Wisconsin dairy farms is 124 cows, the industry is highly segmented by herd size. Dairy farms enrolled in this project were comparable to larger herds enrolled in other studies conducted in this region (Oliveira et al., 2013; Rowbotham and Ruegg, 2015). Milk yield and bulk tank SCC of enrolled herds were similar to previous studies conducted in this region (Oliveira et al., 2013; Rowbotham and Ruegg, 2015). The farms enrolled in this study used a variety of milking systems and various types of teatcup liners (Table 1), but all used narrow-bore vented liners typical of those commonly used on North American dairy farms. Previous studies that have measured teat dimensions have included single farms (Guarín and Ruegg, 2016) and multiple farms (Tilki et al., 2005; Zwertvaegher et al. 2013b) or were conducted using multiple farms but relied on historical data or on information collected by farmers (Seykora and McDaniel, 1986). Enrollment in our study included cows from 9 herds with the objective of minimizing herd effects and broadening the potential reference population. Enrolled farms used different types of milking equipment and likely had greater genetic variability compared with a single farm.

The distribution of parities of cows on larger dairy farms in our region is typically skewed toward younger animals, and cows enrolled in this study had an average of 2.0 lactations as compared with a previous study (Guarín and Ruegg, 2016) in which the average parity was 2.6. Characteristics of teats of cows enrolled in this study were similar to characteristic of teats of primiparous cows previously measured at the University of Wisconsin research farms (Guarín and Ruegg, 2016). Length and width of the teats are known to increase with parity (Seykora and McDaniel, 1986; Tilki et al., 2005; Guarín and Ruegg, 2016). Approximately, 30 years ago Seykora and McDaniel (1986) measured teat length and barrel diameter of 5,934 Holstein cows across several farms. Teat lengths were 50 and 42 mm (first-lactation cows), 53 and 44 mm (second-lactation cows), 55 and 46 mm (third-lactation cows), 56 and 46 mm (fourth-lactation cows), and 56 and 48 mm (fifth-lactation cows), for front and rear teats, respectively. As compared with these historic measurements, teats measured in this study were much shorter and illustrate how selection for shorter teats has dramatically changed teat dimensions. Similar to Zwertvaegher et al. (2012), we confirmed that quarter position (front versus rear), parity, and stage of lactation have important associations with teat dimensions.

In this study, milk samples were obtained from mammary gland quarters rather than composite milk. Composite milk samples are often used to diagnose subclinical mastitis (Reyher and Dohoo, 2011). However, quarter milk samples have greater sensitivity than composite milk samples and accurately reflect inflammation that defines subclinical mastitis occurring at the quarter level (Nielsen et al., 2005; Reyher and Dohoo, 2011). Measurement of SCC using quarter-milk samples was necessary because the experimental unit of our study was the quarter, thus allowing us to relate teat dimensions to QSCC.

Parity and DIM were significantly associated with QSCC. Both of these factors are well known to be associated with increased risk for mastitis. Host-related factors have an important role in increasing susceptibil-

Table 2. Descriptive characteristics of teats enrolled in the study classified by teat position (front or rear) and by farm

Farm	Teat position	No. of teats	Teat length (mm)		Teat barrel diameter (mm)		Teat apex diameter (mm)		QSCC[1] × 1,000 cells/mL		QSCC >150,000 cells/mL (%)
			Mean	SD	Mean	SD	Mean	SD	Median	Range	
A	Front	230	41.2	7.7	22.8	2.9	20.3	1.9	33.5	4–6,024	20.5
	Rear	227	36.1	6.5	21.7	2.4	19.1	2.0	32.0	3–9,999	20.5
B	Front	188	45.2	7.2	22.4	2.6	18.3	2.5	39.0	3–6,342	20.2
	Rear	188	38.4	6.3	21.4	2.3	17.5	2.5	34.0	5–6,597	21.7
C	Front	229	48.4	9.4	22.6	2.7	19.1	2.1	37.0	4–4,466	18.9
	Rear	234	41.9	7.3	22.1	2.4	18.5	2.0	36.0	5–9,999	22.3
D	Front	241	45.1	9.0	22.0	2.5	19.1	1.9	32.0	6–9,999	19.8
	Rear	252	37.1	6.5	20.8	2.0	18.1	2.0	33.0	6–9,999	21.3
E	Front	210	43.5	7.9	22.8	2.8	19.8	2.1	30.5	6–9,999	20.0
	Rear	220	37.6	6.4	22.1	2.7	18.8	2.1	31.5	6–9,999	18.3
F	Front	179	46.6	7.0	22.5	2.6	18.9	2.1	27.0	4–9,999	16.7
	Rear	180	38.9	5.9	21.3	1.9	17.7	2.2	27.0	6–9,999	20.0
G	Front	195	47.4	7.4	22.1	3.0	19.1	2.4	20.0	3–1,342	16.0
	Rear	199	41.8	7.4	21.7	2.3	18.8	2.1	22.0	2–9,999	12.0
H	Front	166	45.3	8.9	21.6	2.9	18.8	2.4	63.0	8–6,786	27.8
	Rear	172	38.2	7.6	21.2	2.7	18.2	2.3	81.5	5–7,982	31.8
I	Front	202	46.8	8.8	23.4	3.1	19.8	2.0	50.0	4–9,999	27.9
	Rear	201	39.9	7.5	22.9	2.4	19.4	2.0	53.0	6–9,999	30.4
Total	Front	1,840	45.4***	8.5	22.5	2.8	19.3***	2.2	35.0**	3–9,999	20.7
	Rear	1,873	38.8	7.1	21.7	2.4	18.5	2.2	33.0	2–9,999	21.8

[1] QSCC = quarter-level SCC (cells/mL).

**, ***Significant difference from rear quarters: **$P < 0.05$; ***$P < 0.001$.

Table 3. Models [2] and [3]: Final mixed models for effect of teat dimensions on $\log_{10}$ quarter (Q)SCC by teat position (front or rear, n = 1,789 and n = 1,823 quarters for models [2] and [3], respectively, on 9 farms)

Predictor	Coefficient β	SE (β)	*P*-value	Covariance parameter estimates
Model [2]—front quarters				
Intercept	1.365	0.16	<0.001	
DIM	0.002	0.00	<0.001	
Parity			<0.001	
1	Referent			
2	0.214	0.04	<0.001	
≥3	0.204	0.04	<0.001	
Teat length	−0.001	0.00	0.768	
Teat barrel diameter	−0.013	0.01	0.076	
Teat apex diameter	0.024	0.01	0.005	
Farm				0.009
Cow(farm)				0.144
Compound symmetry—quarter(cow)				0.007
Residual				0.207
Model [3]—rear quarters				
Intercept	1.556	0.18	<0.001	
DIM	0.002	0.00	<0.001	
Parity			<0.001	
1	Referent			
2	0.132	0.04	0.003	
≥3	0.164	0.05	<0.001	
Teat length	−0.004	0.00	0.143	
Teat barrel diameter	−0.017	0.01	0.077	
Teat apex diameter	0.014	0.01	0.156	
Farm				0.009
Cow(farm)				0.169
Compound symmetry—quarter(cow)				0.003
Residual				0.240

ity to mastitis (Sordillo et al., 1997). Parity has been associated with increased risk of mastitis in numerous studies (Barkema et al., 2006; Pinzón-Sánchez and Ruegg, 2011; Watters et al., 2013). Increased DIM is associated with increased exposure to mastitis pathogens, and increased parity is often related to decreased resistance to infection. As cows age, the udders develop more depth and greater contact with environmental pathogens is possible.

Quarter position was associated with QSCC, and milk from front quarters had slightly lesser QSCC compared with rear quarters. The present results are

Table 4. Model [5]: Final mixed model for effect of teat hyperkeratosis on $\log_{10}$ quarter (Q)SCC (n = 3,592 quarters on 9 farms)

Predictor	Coefficient β	SE (β)	*P*-value	Covariance parameter estimates
Intercept	1.677	0.12	<0.001	
Hyperkeratosis score[1]			0.001	
N	Referent			
S	−0.060	0.03	0.039	
R	−0.063	0.05	0.227	
VR	0.341	0.12	0.003	
DIM	0.002	0.00	<0.001	
Parity			<0.001	
1	Referent			
2	0.173	0.04	<0.001	
≥3	0.187	0.04	<0.001	
Farm				0.009
Cow(farm)				0.138
Compound symmetry—quarter(cow)				0.003
Residual				0.244

[1] Hyperkeratosis scores: N = no ring; S = smooth or slight ring; R = rough ring; VR = very rough ring.

in agreement with the results of Barkema et al. (1997) who observed less frequent increased levels of SCC in front quarters compared with rear quarters. However, it should be noted that the herds enrolled in our study were considerably different than those enrolled by Barkema et al. (1997). In our study, the average number of lactating cows per farm was 1,424, whereas it was 61 in the European study (Barkema et al., 1997). As expected for the time period and herd type, Barkema et al., (1997) reported that *Staphylococcus aureus* was the most frequent cause of mastitis for cows enrolled in that study. Although etiology was not determined for quarters enrolled in our study, the prevalence of mastitis caused by *Staph. aureus* is typically quite low for large Wisconsin dairy farms and for many years the most frequent isolates are environmental pathogens (Makovec and Ruegg, 2003; Oliveira et al., 2013). The similarities between results of Barkema et al. (1997) and our study suggest that possible differences in QSCC by teat position (front or rear) occur with different exposures to pathogens and other risk factors associated with differences in farm size. Future work should investigate possible interactions between risk of mastitis relative to quarter position and bedding types.

In this study, the models for front and for rear quarters demonstrated that narrower teat barrels tended to be associated with increased QSCC. This finding was unexpected and is difficult to explain. Associations between wider teat barrels and subclinical mastitis have been previously described (Zwertvaegher et al., 2013b). In their experiment, Zwertvaegher et al. (2013b) measured 72 Holstein-Friesian cows on 6 farms with an average milk production of 9,368 kg of milk per cow per year. The DIM and parity distribution in Zwertvaegher et al. (2013b) was similar to our study. Zwertvaegher et al. (2013b) reported that QSCC was greater in milk obtained from teats with greater changes in the barrels between pre- and postmilking compared with SCC of milk from teats that had less milking machine–induced change in their barrel size. In our study, only premilking teat characteristics were evaluated because our previous study (Guarín and Ruegg, 2016) demonstrated that milking machine–induced changes were not significantly associated with clinical mastitis. We observed a tendency for QSCC to decrease as teat barrel diameter increased. These apparently contradictory associations were possibly related to differences in the type of liners used most commonly in Europe (medium-bore liners) in contrast to the narrow-bore liners used by herds enrolled in the current study.

In a companion case–control study that enrolled cows from a single university research farm (Guarín and Ruegg, 2016), we demonstrated that cows had a 20% increased risk of clinical mastitis for each 1-mm increase in the diameter of the front teats apexes, but increased risk of clinical mastitis was not observed for rear teats. In our current study, the positive association of teat apex diameter with increased QSCC was also observed only for front teats. Front teats were longer and wider than rear teats, as described previously others (Seykora and McDaniel, 1986; Tilki et al., 2005; Guarín and Ruegg, 2016). Cows with teat diameters that were greater than the herd average have previously been reported to have increased risk of mastitis compared with herdmates (Slettbakk et al., 1995). A possible explanation may be that teats with larger diameters tend to have larger teat orifices and wider teat canals (Chrystal et al., 1999; Paulrud, 2005). Seykora and McDaniel (1985) hypothesized that wider teat canals may not have fully functional teat sphincters and cannot efficiently close the teat orifice. Furthermore, wider teat diameters are associated with increased milk flow rates and greater milk production in dairy cows (Seykora and McDaniel, 1985). The herds included in our study were typical of high-producing North American Holstein herds with relatively short teats and very fast milk flow rates (data collected but not shown because of difficulties in confirming accuracy of cow identification and differences in measurements of different milk meter systems used in enrolled farms). Thus, these characteristics are possibly associated with increased risk of intramammary infection.

We did not observe an association of teat shape with QSCC. Early work about teat shape and its association with production characteristics and mastitis was reported by Hickman (1964). This researcher associated funnel-shaped teats (and teats of small diameter) with lower SCC and a decreased frequency of mastitis compared with cylindrical-shaped teats (Hickman, 1964). Chrystal et al. (1999) did not report associations between teat-end shape and somatic cell scores. The authors attributed the lack of association to the use of proper teat sanitation and management practices, which decreased the influence of the teat-end shape on IMI. The same positive milking management practices attributed by Chrystal et al. (1999) may have influenced our results. The median SCC of cows on the test day before the start of the study was well under 100,000 cells/mL, indicating excellent overall management of mastitis.

In the current study, hyperkeratosis scores were associated with QSCC, but a strong association was seen for the most extreme score only. Compared with teats with scores N, teats with scores of VR had greater QSCC. Conversely, teats with scores of S had decreased QSCC; however, the magnitude of the difference was much greater for teats scored VR. Of all studied predictors, a VR score had the greatest effect associated with increased QSCC, demonstrating that this undesirable attribute had a strong influence. These findings are in agreement with Neijenhuis et al. (2001a), who described an association between increased risk of clinical mastitis and very rough teat ends. Although the exact mechanism of how VR teats increase the risk of IMI is not known, teats scored as VR may be more difficult to clean than teats with smoother teat orifices. Colonization of teat skin with bacteria is associated with environmental exposures (Rowbotham, 2015); thus, increased exposure and difficulties in cleaning VR teat ends may increase the opportunities for IMI. Although only 29 out of the 3,713 scores were VR scores, the association of this teat-end damage with QSCC was of great magnitude. Other teat scores did not have as much effect on QSCC as VR scores. Some of the known factors influencing the level of hyperkeratosis of the teat ends are the climate, teat-end shape, teat position, teat length, herd milk production level, lactation stage, and parity (Neijenhuis et al., 2001b; Reinemann, 2012). The researchers identifying these factors also found an association of teat shape and teat-end callosity that was not observed during the current study. That difference might be due to the definition the authors used for shape of the teat ends in contrast with our definition, which was based on the ratio the teat barrel to the teat apex.

Zwertvaegher et al. (2013a) commented that the use of rulers, tapes, and calipers is practical, inexpensive, and simple, although accuracy depends on previous experience using these devices to measure teats. The researchers (Zwertvaegher et al., 2013a) found nonsignificant differences, low coefficient of variation, and strong correlations when they analyzed duplicate measurements and intra-operator variation for the use of rulers to determine teat dimensions. In our study, one researcher performed all teat measurements, and several limitations were noted for the use of rulers under real field conditions. The device may have limitations in dark environments, and its having a self-contained light consequently helped overcome this issue. Because experience with the device is important, the researcher who performed teat measurements (JFG) had previously measured >500 teats. However, cows that exhibited excessive movement or kicking were excluded from the study to ensure accuracy and to protect the safety of researchers. In each case, only about 1 or 2 animals per farm were excluded, and we do not believe that these exclusions meaningfully affected the results of this study. Some selection bias may have occurred by enrolling just cows that entered the first 2 stalls on each side of the parlor each turn. However, we believe that this bias is unlikely because cows were brought to the parlor in groups of 100 to 250 cows and were contained in holding pens while waiting to enter. On most farms, crowd gates were used to encourage cows to enter the parlor, and the first 2 stalls included cows from all parities and stages of lactation.

CONCLUSIONS

In this study, we examined associations between anatomical characteristics of teats and the prevalence of subclinical mastitis at the quarter level. This study helps to clarify the association between mastitis and the diameter of the front teat apexes. Wider teat apexes in front teats were associated with increased occurrence of subclinical mastitis, but that association was not observed for rear teats. No association between teat shape (triangular or squared) and QSCC was found, and associations between teat-end shape and QSCC reported by other researchers are likely related to positive associations between severe teat hyperkeratosis and QSCC. Further research should focus on the association of wide front teat apexes and other risk factors such as wide teat canals, teat-end hyperkeratosis, and increased IMI.

ACKNOWLEDGMENTS

The authors thank the owners and personnel of each of the enrolled dairy farms in this project for their help. The project was partially funded by the government of Colombia and the Departamento Administrativo de Ciencia, Tecnología e Innovación–COLCIENCIAS, Bogota, Colombia, and by unrestricted gift funds of the senior author (PLR), University of Wisconsin, Madison.

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