Research Article

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Preliminary bacterial study on subclinical mastitis and teat condition in dairy herds around Shiraz

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Abstract: Mastitis is a widely occurring and costly disease in the dairy industry. The aim of this study was to isolate bacterial causes of subclinical mastitis and investigate the relationship between subclinical mastitis and teat condition in dairy herds located around Shiraz, Fars province, Iran. From 7 commercial dairy herds around Shiraz 354 lactating cows were selected randomly. Subclinical mastitis was evaluated by the California Mastitis Test (CMT), electrical conductivity (EC), and somatic cell count (SCC). Teat condition was evaluated by measuring teat width and length. In the 68 samples of bacterial cultures identified, 14 different bacterial species were found. *Staphylococcus aureus* and *Streptococcus agalactiae* were the most common isolates with 19 (27.94%) and 14 (20.59%), respectively. There were no significant differences between the width and length of the 4 teats in different CMT reactions. There was, however, significant correlation between CMT reaction and teat width (r = 0.14 to 0.20). In addition, there was significant correlation between SCC and teat injuries (r = 0.13). The difference between number of SCC in CMT positive and negative cows, as well as EC positive and negative cows, was significant. It is concluded that some anatomical characteristics of the teat can affect the udder's susceptibility to mastitis. Therefore, bull selection programs may be targeted to improve mastitis control in dairy cow farms.

Key words: Subclinical mastitis, teat, somatic cell count, *Staphylococcus aureus*, dairy herds

Introduction

Mastitis remains the most common infectious disease in adult dairy cattle and the most costly disease affecting the dairy industry. A major portion of this cost is not attributable to cows with clinically apparent mastitis but, rather, is the result of subclinical mastitis and the associated decrease in milk production (1).

Total milk loss from quarters affected with subclinical mastitis has been estimated to vary from 10% to 26%. Approximately 75% of the economic loss from subclinical mastitis is attributable to loss of milk production. The most significant subclinical abnormality of the milk is the increase in SCC, the most common measure of milk quality and udder health (2).

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Changes in teat tissue around the teat orifice may increase the likelihood of bacterial penetration of the udder and predispose the cow to subclinical mastitis (3). Cow factors including teat-end shape, teat position, teat length, milk yield, stage of lactation, and parity are associated with the degree of teat-end callosity (TEC) (4,5). TEC is influenced by milking machine conditions and milking management (3,6,7). Histological study of teat tissue shows good correlation with TEC scores (8).

Changes in teat-end tissue result from mechanical forces exerted by vacuum and the collapsing liner during machine milking. Rasmussen et al. (9) reviewed the influence of liner type and pulsation on teat condition. When bacteria have passed through the teat canal they may be moved to the quarter cistern or milk ducts by compression of the teat cistern during pulsation. If this happens in the early stages of milking, there is a high probability that those bacteria will be flushed out later during the milking. Mein et al. (10) and Rasmussen (6) focused on the effects of over-milking, pulsation, and the liner on teat condition. They considered the following teat condition parameters: teat swelling, TEC, and keratin. These teat parameters are on a gradual scale and are always influenced by machine milking.

The objective of this study was to compare teat condition and subclinical mastitis in dairy cows around Shiraz.

Materials and methods

Farm locations

This study was carried out on dairy farms around Shiraz, located in southwest Iran at 29°15′N latitude 52°35′E longitude. The climate is hot and dry, and the elevation is 1500 m above sea level.

Animals

From 7 commercial dairy herds 354 lactating cows were examined. Random sampling was used for selecting the cows from the farms visited.

The parity of cows was 2.7, average lactation stage was 155 DIM, and average of milk was 24 kg/day. Cows were kept in semi-confinement open housing and milked 3 times daily. Feeding was based on corn silage, alfalfa, grass silage, and corn meal as a total mixed ration.

Clinical examinations

The quarters and teats of each cow were observed, and every abnormality, such as mammary drop, hyperkeratosis (rough and very rough) in the teat orifice, was recorded. The teat length (end to base) and width were determined by using a roller and caliper before premilking. Moreover, dry quarters were recorded on the recording sheet. Teat-end shape was classified as round, flat, pointed, or inverted (11). Pointed teats have more hyperkeratosis and lesions. Flat teats tend to milk slower, but have fewer infections.

California Mastitis Test

The California Mastitis Test (CMT) reagent (DeLaval, Wroclaw, Poland) and method were used alongside the physical examinations, and the test was carried out as described. Reactions were graded 1, 2, 3, 4, or 5 according to the Scandinavian recommendations (12). Samples for bacteriological culture were collected from cows with a CMT reaction grade of 1 or more.

Electrical conductivity

The electrical conductivity of milk in each quarter was evaluated by a hand-held electrical conductivity meter (Mas-Detector, New Zealand). The Mas-Detector indicates EC negative (green light) and positive (red light) for the presence of subclinical mastitis in each quarter.

Bacterial sampling and culture

Prior to quarter sampling the teat ends were cleaned and rubbed with cotton moistened in 70% alcohol. The 3 initial streams of milk were discarded, and approximately 5 mL of foremilk was collected into sterile 10 mL universal (polythene) tubes kept on ice. A portion of each quarter milk sample was inspected for clots, discoloration, or wateriness before adding the CMT reagent. The rest of the milk was immediately transported, cooled (4 °C), to the Microbiology Laboratory, School of Veterinary Medicine, Shiraz University, for bacteriological examination. Each sample in the laboratory was immediately cultured on sheep blood agar and MacConkey agar (Merck), and incubated at 37 °C for 48 h. Standard biochemical tests were used for the isolation and identification of the isolates (13).

Somatic cell count

The somatic cells in milk from each cow were counted by electronic counter (Fossomatic 90, Foss Electric, Denmark).

Statistical analysis

The data were analyzed by using SAS software, version 9.1. One-way ANOVA, Duncan's multiple range tests, correlation, and t tests were used for the statistical analysis. $P < 0.05$ was considered the level of significance.

Results

There was a significant difference between teat width and SCC of quarters with CMT positive and negative reactions. However, there was no significant difference between teat length in the 2 groups and CMT positive and negative reactions. There was a significant difference between teat length and SCC of quarters with positive and negative EC reactions. The difference between teat width in the EC positive and EC negative group, however, was not significant (Table 1). There was no significant difference between the length of teats and width of teats in the CMT positive reaction without bacterial growth, or in the positive reaction group with bacterial growth (Table 2). Means of SCC in CMT positive quarters (based on bacterial isolation) showed no significant difference (Table 2). There was no significant difference between width and length of the 4 teats in the different CMT reactions in our study (Tables 3 and 4). There was, however, significant correlation between teat width and CMT reaction (left front teat, $r = 0.14$; right front teat, $r = 0.15$; left rear teat, $r = 0.20$; and right rear teat, $r = 0.19$). Table 5 shows the bacterial species and their percentages. The mean of SCC in normal cows and cows with one kind of teat abnormality (hyperkeratosis, laceration, conical-shaped teat, flat, funnel, and no center orifice) is shown in Table 6.

Table 1. Comparison of teat width (mean ± SE), length (cm), and SCC ($\times 10^3$) between CMT and EC positive and negative cows.

Test result / Character	CMT negative (n = 230)	CMT positive (n = 115)	EC negative (n = 216)	EC positive (n = 129)
Teat width	2.90 ± 0.027^a	3.06 ± 0.059^b	2.96 ± 0.03	2.94 ± 0.05
Teat length	6.04 ± 0.07	6.21 ± 0.105	5.86 ± 0.067^a	6.24 ± 0.09^b
SCC ($\times 10^3$)	175.62 ± 30.55^a	878.54 ± 123.72^b	315 ± 44.51^a	571.03 ± 107.39^b

Values with different superscripts in each row are those that differ significantly for each test.

Table 2. Comparison of teat width, teat length, and SCC of cows with negative CMT reaction and cows with positive reaction (with or without bacterial growth) (mean ± SE).

Test result / Characters	CMT negative (n = 230)	CMT positive without growth (n = 27)	CMT positive with growth (n = 62)
Teat width	2.90 ± 0.027^a	3.04 ± 0.10^b	3.08 ± 0.095^b
Teat length	6.04 ± 0.07	6.19 ± 0.19	6.09 ± 0.16
SCC ($\times 10^3$)	175.62 ± 30.55^a	721.44 ± 235.06^b	837.47 ± 127.25^b

Values with different superscripts in each row are those that differ significantly.

Table 3. Comparison of teat width (cm) in different groups of CMT reaction (mean ± SE).

Teats / CMT result	Left front	Right front	Left rear	Right rear
Normal	2.70 ± 0.06	2.71 ± 0.06	2.67 ± 0.06	2.69 ± 0.06
Trace	2.98 ± 0.03	2.97 ± 0.03	2.98 ± 0.03	2.96 ± 0.03
+	2.96 ± 0.07	3.02 ± 0.07	3.01 ± 0.06	2.97 ± 0.11
++	3.23 ± 0.22	3.15 ± 0.28	3.00 ± 0.12	3.02 ± 0.14

Table 4. Comparison of teat length (cm) in different groups of CMT reaction (mean ± SE).

Teats / CMT result	Left front	Right front	Left rear	Right rear
Normal	6.00 ± 0.16	6.00 ± 0.15	6.00 ± 0.15	6.03 ± 0.15
Trace	6.04 ± 0.06	6.02 ± 0.06	6.09 ± 0.07	6.08 ± 0.07
+	6.44 ± 0.18	6.50 ± 0.16	6.29 ± 0.16	6.16 ± 0.16
++	6.00 ± 0.72	5.42 ± 0.89	5.58 ± 0.35	5.87 ± 0.58

Table 5. Number and percentage of bacterial isolates cultured from quarters affected by subclinical mastitis in dairy cows around Shiraz.

Bacterial species	Number	Percentages %
Staphylococcus aureus	19	27.94
Streptococcus agalactiae	14	20.59
Streptococcus dysgalactiae	7	10.29
Corynebacterium bovis	5	7.35
Staphylococcus epidermidis	4	5.88
Escherichia coli	3	4.41
Bacillus lentus	3	4.41
Micrococcus spp.	3	4.41
Enterococcus faecalis	3	4.41
Bacillus cereus	2	2.94
Corynebacterium psudotuberculosis	2	2.94
Lactobacillus spp.	1	1.47
Streptococcus bovis	1	1.47
Corynebacterium renale	1	1.47
Total	68	100

Table 6. Comparison of SCC ($\times 10^3$) in different teat injuries (mean ± SE).

Injuries	Normal cows (n = 280)	Abnormal cows (n = 74)
SCC ($\times 10^3$)	372.67 ± 57.20	659.01 ± 120.17*

*P < 0.05; values with different superscripts in each row are those that differ significantly.

The mean of SCC in cows with abnormalities was significantly higher than SCC in normal cows. The SCC numbers of left and right front quarters differed significantly between CMT reaction groups (Table 7). Furthermore, correlation between SCC of positive CMT tests and the SCC numbers from the same quarters was positive ($r = 0.36$, $P < 0.05$). Our results showed that wider and longer teats have more SCC, but the differences between groups are not significant (Table 8).

Discussion

Bacterial isolation

In this study we isolated 8 various genera of bacteria from all samples that were collected for bacteriological culture to detect subclinical mastitis agents. Our results indicated that 55.8% of isolated bacteria were contagious mastitis agents, and *Staphylococcus aureus* was the most significant bacterium (27.9% of all isolated bacteria). Some

Table 7. Comparison of means (±SE) of SCC ($\times 10^3$) in different CMT results from 4 teats.

Quarters / CMT	Left front SCC (×1000)	Right front SCC (×1000)	Left rear SCC (×1000)	Right rear SCC (×1000)
Normal	11.69 ± 2.79^{b}	70.86 ± 18.25^{c}	112.36 ± 43.88	114.03 ± 42.86
Trace	867.77 ± 3.38^{ab}	323.26 ± 52.69^{abc}	292.48 ± 39.89	338.95 ± 54.07
+	1275.34 ± 32.74^{ab}	1004.94 ± 193.15^{abc}	853.96 ± 188.53	837.7 ± 181.81
++	1363.93 ± 194.54^{a}	1523.50 ± 795.41^{a}	1389.22 ± 380.59	1646.63 ± 382.15

Values with different superscripts in each column are those that differ significantly.

Table 8. Comparison of teat length and width between cows with different SCC.

SCC ($\times 10^3$)	Teat length	Teat width
<100 (n = 171)	5.95 ± 0.07	2.89 ± 0.03
100-500 (n = 112)	6.18 ± 0.10	2.99 ± 0.06
500-1000 (n = 25)	6.26 ± 0.20	2.96 ± 0.05
>1000 (n = 37)	6.37 ± 0.21	3.10 ± 0.08

reports indicate that *S. aureus* is one of the most prevalent causes of intramammary infection (IMI) and estimate that 7% to 40% of all cows are infected (14).

It is widely accepted that most contagious mastitis is transmitted by fomites from infected cows to non-infected herd mates during the milking process. Milkers' hands, the milking unit, and udder wash cloths have been implicated as primary fomites. Evidence also suggests that flies may be fomites for *S. aureus* IMI and that *S. aureus* may be transmitted by aerosols. However, these are of secondary importance in the transmission of contagious IMI (15).

In this study 44.2% of isolated bacteria were environmental mastitis agents, and the most prevalent bacterium was *Streptococcus dysgalactiae*. Among the collected samples, 20.2% did not show any growth. Along with improvement in contagious mastitis control programs (post-milking teat dipping, total dry cow therapy, culling, therapy of clinical mastitis cases, and proper maintenance of milking equipment), the relative importance of environmental mastitis increased in dairy herds (16). The rate of occurrence of clinical mastitis cases in 9 well-managed herds was reported to be 45.7 cases per 100 cows over 305 days. Causes of clinical cases were coliforms (29.7%), environmental streptococci (25.4%), bacteriologically negative (27.2%), and other causes (17.6%). Contagious pathogens were isolated from only 3.45% of cases (17). Despite this, our results show that contagious mastitis remains a serious problem in this region. Furthermore, this study highlights the need for increased focus on milking hygiene. In our study 117 cows out of 354 (33%) had CMT positive reaction and subclinical mastitis (15.5% of all quarters).

Teat length and diameter

Our data showed a significant interdependence between CMT positive and high SCC and teat width. However, these study results did not reveal any relationship between teat length and CMT reactions. Rogers and Spencer (18) have shown that variation among cows in machine linear slips and manual adjustment within and across lactation and days in milk (DIM) can be partially explained by udder and teat morphology. Wider teats were associated with increased liner slips and increased manual adjustment. In addition, larger teat diameters and longer teats tended to be associated with increased liner slips. Weiss et al. (19) found no correlation between milkability traits and externally measurable teat characteristics, such as teat length or teat diameter.

Anatomical characteristics, such as teat canal length and teat length and width, as well as functional traits (e.g., milk flow profiles and teat condition) of the teat, are presumed to have considerable influence on milkability (19).

Teat diameter did not differ between front and rear teats. Teat shape in general did not increase the risk of milk leakage in either experimental group (20,21). Klaas et al. (20) were determined that teat canal protrusions and inverted teat-end shapes were risk factors for leakage. The slightly shorter teat canals could be partially responsible for the occurrence of milk leakage. The main problem related to teat canal length is its influence on the passage of pathogens through the canal, and thus its susceptibility to IMI (22).

Rathore and Sheldrake (23) reported that teats of larger diameter had larger orifices. Part of the correlation between teat orifice and mastitis may be due to older cows having both larger teats and more relaxed sphincter muscles (24).

The average evaluation of teat length of the Black-and-White cows was near the optimal; consequently, teat length and teat thickness had no impact on SCC in milk. Juozaitiene et al. (25) reported the negative relationship between SCC and milk yield during lactation and the relationship between somatic cell count and udder or teat morphology. Reducing milk SCC through the selection of Black-and-White cows based on udder and teat morphology can improve milk quality.

Teat shape and shape of teat end

In our study we observed 5 different teat injuries. The mean of SCC in cows with teat injuries was higher than SCC in normal cows. In our study there was significant correlation between SCC and teat injury. Mulei (26) examined the mammary gland quarters of 139 lactating dairy cows from small-scale dairy herds. He showed that the mammary gland quarters with teat lesions were 7.2 times more likely to have a positive CMT (P < 0.01) and 5.6 times more likely to have bacterial organisms (P < 0.01) isolated from them than those without any lesions.

Rathore (27) reported that cows with funnel-shaped teats gave 10.9% more milk and had lower somatic cell counts (208,000 cells/mL vs. 441,000 cells/mL) from data on 548 Friesians in New South Wales. Funnel-shaped teats offered greater resistance to being drawn into the teat cup and appeared to milk out more completely. Neijenhuis et al. (5) reported that teat-end shape was associated with the degree of teat-end callosity thickness (TECT) and teat-end callosity roughness (TECR). With increasing peak yield, TECT increased. Teat length was not associated with TEC. The risk of subclinical mastitis was highest for cows with long and thick teats. The risk of subclinical mastitis for cows with funnel-shaped teats was found to be lower than for cows with cylindrical teats. The risk of subclinical mastitis was found to increase as parity rose (28). Bakken (4) found that funnel-shaped teats had a slightly higher incidence of *S. aureus* mastitis.

Bakken (4) also found no correlation between teat-end lesions and incidence of clinical or subclinical mastitis. Further studies did not detect a relationship between SCC and teat-end shape (4,29). Chrystal et al. (11) found that round teat ends accounted for more than 50% of teat-end shapes; therefore, frequencies of other teat-end shapes were low, which might have resulted in a low estimation of effects for these teat-end shapes.

Neijenhuis et al. (30) speculated that a small amount of TEC does not appear to increase the risk of intra-mammary infection in lactating dairy cows and may be a beneficial physiological response of the teat to machine milking. A greater degree of TEC and roughness is associated with an increased probability of new intra-mammary infections. General consensus is that there seems to be little relationship between lesion score and mastitis, unless the lesion is acute with an ulcerated or hemorrhagic appearance.

In conclusion, contagious mastitis agents are the most prevalent cause of IMI in this region, and *Staphylococcus aureus* is the most significant bacterium. These findings emphasize the need to pay more attention to milking and milking machine hygiene in this region. Anatomical characteristics of the teat, such as teat width, have significant influence the on occurrence of subclinical mastitis. Bull selection programs can be used to improve mastitis control programs on dairy cow farms. Our results show that cows with teat injuries have more SCC. The most prevalent injury in this region is hyperkeratosis, and conical-shaped teats have the highest SCC.

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