Haryana Vet. (April, 2021) **60(SI)**, 5-9 **Research Article**

ASSOCIATION BETWEEN TEAT-END HYPERKERATOSIS AND UDDER HEALTH IN CROSSBRED DAIRY COWS

DIVYA TRIVEDI[1], RAJ SUKHBIR SINGH*and DHIRAJ KUMAR GUPTA[1]
[1]Department of Veterinary Medicine, Department of Teaching Veterinary Clinical Complex,
College of Veterinary Science,
Guru Angad Dev Veterinary and Animal Sciences University,Ludhiana–141 004, India

Received: 24.04.2020; Accepted: 10.08.2020

ABSTRACT

A longitudinal study was carried out to observe the occurrence of teat-end hyperkeratosis (TEH) and its association with milk somatic cell count (SCC) and subclinical mastitis (SCM). A total of 30 Holstein Friesian × Sahiwal crossbred dairy cows in their first 7 months of lactation were randomly selected. Each month, teat-ends were evaluated for severity of TEH and scored from 1 to 4. Also, quarter foremilk samples were collected monthly for estimation of SCC. During the lactation, overall mean TEH score was 1.42 ± 0.64 with 66% teats showing TEH score of 1 (normal); only 7.1% teats had TEH score of 3 (rough) and 4 (severely rough). Mean TEH score increased significantly (P<0.001) as the lactation advanced, with higher occurrence in front quarters (P<0.001) as compared with rear quarters. Mean SCC was significantly associated with rough to severely rough TEH score (P<0.01). Teats with rough to severely rough TEH score were identified as a risk for SCM (OR:5.77; CI:2.23-14.95; P<0.001). In conclusion, teats with higher TEH score poses significant risk for poor udder health in Holstein Friesian × Sahiwal dairy cows, therefore, emphasizing regular monitoring of teat-ends for TEH to evaluate mastitis risk and milking machine related factors.

Keywords: Crossbred cows, Somatic cell count, Subclinical mastitis, Teat-end hyperkeratosis

The teat-end structures, such as teat canal and teat orifice, are the first line of defense against penetration of microorganisms into the udder (Pantoja *et al.*, 2020). The alterations at the teat-ends, especially due to poor milking techniques, are considered as important contributing factors influencing the occurrence of clinical and subclinical mastitis (Mein *et al.*, 2001; Zucali *et al.*, 2009; Pantoja *et al.*, 2020). Teat-end hyperkeratosis (TEH) is considered as one of the teat tissue changes and is defined as the thickening of the skin around the teat orifice due to the formation of a smooth or rough keratin ring (Pantoja *et al.*, 2020). These changes are due to hyperplasia of stratum corneum caused by mechanical forces applied to the teat tissue during machine milking. Development of TEH is a slow process and it may take several weeks for the TEH to become obvious (Neijenhuis *et al.*, 2000; Mein *et al.*, 2001).In addition to milking machine related factors, quarter and cow level factors such as quarter position and morphology, parity, stage of lactation, and milk yield are also possible factors associated with TEH (Zucali *et al.*, 2009; Mitev *et al.*, 2012; Singh *et al.*, 2013; Pantoja *et al.*, 2020). Further, studies have shown an association of TEH with somatic cell counts (SCC) and IMI (Zucali *et al.*, 2009; Mitev *et al.*, 2012).

Evaluation of teat tissue changes over the lactation and its relation to udder health is important for development of control and prevention strategies for mastitis. Therefore, present study was performed to investigate the occurrence of TEH and its association with milk SCC and SCM in Holstein Friesian × Sahiwal dairy cows during the first 7 months of lactation.

*Corresponding author: rsbs_66@rediffmail.com

MATERIALS AND METHODS

Animals and farm management: The study was conducted at dairy farm of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India. A total of 30 recently calved apparently healthy Holstein Friesian×Sahiwal crossbred dairy cows in their 1st (n=11) or 2nd (n=19) parity were included in the study. Early parity cows were selected in order to minimize the risk of infections as well as milking machine induced teat tissue changes from previous lactations. Cows were milked twice daily (4.00 AM and 3.00 PM). Farm has a DeLaval low pipeline milking system with 6+6 parallel tandem type milking parlor. Milking machines were equipped with electronic milk meter (DeLaval) and worked at vacuum level of 42 kPa, pulsation rate of 60 pulsations/min and pulsation ratio of 65/35. Clusters were removed automatically once milk flow rate fell below 300 g/min with a delay of 20 sec. The overall test day afternoon milk yield (Mean ± SD) of the selected cows was 5.46 ± 1.86 kg. Liners were replaced every 6 months. Routine milking procedure included cleaning of teats with water, followed by drying off with clean cloth, pre-stripping and application of cluster within 60-90 sec, and after completion of milking, post-milking teat disinfection was done using commercially available teat-dip.

Sampling and analysis: Each cow was observed at monthly interval for 1st seven months of lactation. Quarter foremilk samples were collected at each visit. Before collection of samples, proper cleanliness and dryness of teats was ensured. Teat-ends were disinfected with cotton swabs drenched in 70% alcohol. Subsequently, the 1st few streams of milk were discarded, and approximately 20 ml of individual quarter foremilk samples were collected in sterilized test tubes. Milk samples were packed in ice box and transferred to the mastitis laboratory for further analysis. At the end of milking and immediately after cluster removal, the same cows were evaluated for TEH using teat-end examination. This evaluation was done using a scoring method (scores 1 to 4) as described by Mein *et al.* (2001); where score 1= a healthy teat without a ring around the teat orifice; score 2 = a slightly raised white smooth ring around the teat orifice; score 3 = a raised and roughened keratin ring, extending 1-3 mm with visible old keratin; and score 4 = a severely roughened keratin ring, extending 4 mm or more from the teat orifice with clearly visible old keratin. All the observations were conducted by the same person using reference photographs (Fig. 1).

In the laboratory, after thawing, quarter fore milk samples were subjected to culture as per the standard microbial procedures of the National Mastitis Council (Hogan *et al.*, 1999). Somatic cells were estimated using an automatic cell counter (DELTA Instrument, BV Kelvinlaan 3, 9207 JB Drachten, Netherland). Quarter health was defined on the basis of results of culture (culture positive or negative) and SCC (< 200, 000 cells/ml or ≥ 200, 000 cells/ml) of quarter foremilk samples. The udder quarter was classified as healthy when SCC was < 200, 000 cells/ml and culture result was negative (no bacteria was isolated). Latent mastitis quarters had SCC < 200, 000 cells/ml but culture positive (one or more bacteria were isolated). Non-specific mastitis quarters had SCC ≥ 200, 000 cells/ml but culture negative, and specific mastitis quarters had SCC ≥ 200, 000 cells/ml and culture positive.

Statistical analysis: For the purpose of statistical analysis, some of the variables were categorized as described below:

Quarter health: Healthy group included both healthy and latent mastitis quarters since it is generally assumed that latent infection induce no significant inflammatory reaction in udder; and SCM group included both non-specific and specific mastitis quarters.

Teat-end hyperkeratosis: Normal (TEH score = 1), Smooth (TEH score = 2), and Rough to severely rough (TEH score = 3 and 4).

Quarter position: Front (left and right front quarters) and Rear (left and right rear quarters).

Stage of lactation: 30 days, 60 days, 120 days, 180 days and 210 days of lactation.

Data were stored in Microsoft Excel and analyzed by MINITAB statistical software (release 14.2). Frequencies of categorical variables, prevalence of TEH scores and means, standard deviation of means, minimum and maximum values of continuous variables were calculated. Somatic cell scores (SCS) were calculated from SCC by natural logarithmic transformation (LnqSCC) in order to obtain the distribution close to normal. Data collected during the first 7 months of lactation were analyzed by ANOVA method. Results were expressed as statistically significant at $P < 0.05$. Further, binary logistic regression was performed to study the association of SCM (occurrence of SCM quarters compared with healthy quarters) and TEH (normal to smooth =1+2 vs. rough to severely rough =3+4). Variables like stage of lactation and quarter position were also included in the model. The degree of association between response variable and predictor variable was assessed using odds ratio (OR) and confidence interval (95%). Results were expressed as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Of the 840 quarter observations during the entire study, 38 observations (5 each at 1st, 2nd, 3rd and 7th month; and 6 each at 4th, 5th and 6th month of lactation) were excluded from the final data set for various reasons like non-availability of foremilk samples or laboratory analysis results, and quarters became infected with clinical mastitis. So, 802 quarter observations were available for final data analysis. Total observations pertaining to front and rearquarters were 404 and 398, respectively. Most of the quarters were healthy (49.4%) followed by non-specific mastitic quarters (24.8%), specific mastitic quarters (21.9%) and latent mastitic quarters (3.9%).

During the 1st 210 days of lactation, most of the teats had a TEH score 1 (66%; normal) followed by 2 (26.9%; smooth), 3 (6.4%, rough) and 4 (0.7%, severely rough. Overall, only 7.1% teats were in a TEH category of rough to severely rough (TEH scores 3 + 4). The average TEH score was found to be 1.42 ± 0.64. Overall, we observed higher prevalence of teats with normal to smooth TEH score, a finding corroborates with one of the previous studies which also reported higher prevalence of teat with smooth TEH score (41.2%) followed by teats with normal (34.7%) and teats with rough to very rough (24.1%) TEH score (Singh *et al.*, 2013). However, in the present study, teats with rough to severely rough TEH score (sum of scores 3 and 4) were comparatively less (7.1%) frequently observed than those reported by Singh *et al.* (2013) and the average TEH score was also low (1.42 ± 0.64). Probably, this could be due to the reason that all cows involved in this study were either in 1st or 2nd parity. Additionally, the prevalence of teats with poor TEH score at the beginning of the study was considerably low (i.e. only 1.75% teats had rough to severely rough TEH score until 60 days of lactation). In a study conducted by Neijenhuis *et al.*

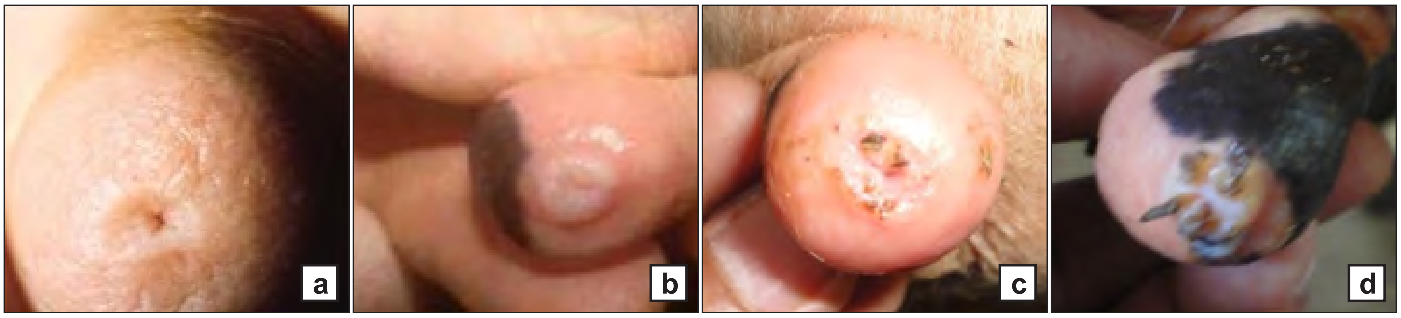

Fig. 1. **(a)** TEH score 1 No ring; **(b)** TEH score 2 Smooth ring; **(c)** TEH score 3 Rough ring; **(d)** TEH score 4 Very rough ring to open lesions or scabs

(2000), 1st lactation cows were shown to have less callused teats than those of older cows (parity >2). Possibly, 1st or 2nd parity cows produce less milk than the multiparous cows, (as observed in the present study, i.e. the test day afternoon mean milk yield of the included cows ranged from 4.25 kg to 6.45 kg) and therefore, have been under less exposure to milking machine action. It has been observed that high-yielding cows with long machine-on times often develop a higher degree of TEH; hence, the duration of applied pressure on the teats is comparatively longer than that of in low-yielding cows with shorter machine-on times. Furthermore, high-yielding cows often have higher milk flow rates which results into increased shear forces affecting the teat canal. This leads to excessive stress for the teat tissue and an increased risk for teat tissue damage and hyperkeratosis (Besier *et al.*, 2016).

Table 1 summarizes the effect of stage of lactation and quarter position on TEH severity. The average TEH score increased significantly ($P < 0.001$) from 1.08 ± 0.27 (day 30) to 1.85 ± 0.74 (day 210) as the lactation advanced. Further, the occurrence of TEH score was significantly ($P<0.05$) higher in front quarters (average TEH score $=1.45 \pm 0.65$) compared with rear quarters (average TEH score $=1.39 \pm 0.64$). Stage of lactation and teat position are likely to affect TEH severity. The risk for development of poor TEH increased as the lactation advanced. Our results are similar to other studies. Shearn and Hillerton (1996) observed highest severity of TEH in mid lactation cows with a decline towards the end of lactation. Neijenhuis *et al.* (2000) while relating development of teat-end callosity thickness with respect to stage of lactation observed a significant increase in the callosity thickness, mainly during the 1st 6 to 8 weeks of lactation. Zucali *et al.* (2009) recorded teat apex scores in primiparous dairy cows up to 1st 120 days of lactation and concluded that as the lactation progressed, teat apex scores increased significantly. The reason for increase in TEH score could be due to the combination of the milk yield, the pressure in the teat skin during milking and the amount of time the pressure is applied. Obviously, cows with higher milk yields have longer machine-on times (Besier *et al.*, 2016). Rysanek *et al.* (2001) suggested that the intense extraction of milk during machine milking initiates a loss of keratin layer. The latter leads to overproduction of keratin from keratinocytes which results in the formation of a keratin ring around the teat orifice. Front teats had significantly ($P<0.05$) higher TEH score (average score $= 1.45 \pm 0.65$) compared with rear teats (average score $= 1.39 \pm 0.64$). Similar to the present study, Sterret *et al.* (2012) also reported significantly higher average TEH score in right and left front teats (1.58 ± 0.09 and 1.62 ± 0.09, respectively) as compared with right and left rear teats (1.37 ± 0.09 and 1.36 ± 0.09, respectively). Probably, this could be explained by the fact that front teats produce less milk, so milking is finished earlier than in the rear teats, and hence, tend to be over-milked for a longer period of time and contributes to TEH (Mein *et al.*, 2001). Probably, the average vacuum level remains higher during this over-milking period as there is no milk flow to dissipate system vacuum and this may result in severe TEH (Hillerton *et al.*, 2001). However, Neijenhuis *et al.* (2000) did not found significant difference in teat-end callosity thickness between front and rear teats from cows in older parity (>2 parity), but 1st and 2nd parity cows showed more callus formation in front teats than in the rear teats. In contrast, Mitev *et al.* (2012) reported higher proportion of TEH score of 3 and 4 (41.2% and 1.2%, respectively) in rear teats than front ones (28.8% and 0%, respectively).

The association of SCS and TEH score, stage of lactation and quarter position is also depicted in table 1. Mean SCS was significantly ($P<0.001$) higher (5.83 ± 0.46 cells/ml) in quarters with rough to severely rough TEH score as compared with quarters with TEH score of 1 and 2 (5.16 ± 0.46 and 5.37 ± 0.47 cells/ml, respectively). A positive correlation was also found between SCS and TEH score ($r = 0.36$, $P<0.001$). It was observed that cows with severe TEH were more likely to have higher SCC compared to cows with normal or smooth TEH (Pantoja *et al.*, 2020). This association indicates an adverse effect of TEH on udder health of dairy cows. Zucali *et al.* (2009) found a significant association between higher TEH score and milk SCC. Studies conducted by Neijenhuis *et al.*

Table 1

Descriptive summary as well as analysis of variance results for the effect of variables studied up to 1st seven months of lactation in 30 crossbred dairy cows

Variable	n (Quarters)	TEH[1] score	LnqSCC[2] (cells/ml)	Absolute SCC (×103 cells/ml)
			Mean ± SD	
TEH score				
Normal	529	NA3	5.16±0.46[C]	260.6±392.3
Smooth	216	NA	5.37±0.47[B]	476.9±884.7
Rough to severely rough	57	NA	5.83±0.46[A]	1106±1320.0
Stage of lactation (days)				
30	115	1.08±0.27[d]	5.11±0.40[C]	232.8±449.9
60	115	1.10±0.32[d]	5.03±0.39[C]	177.7±319.8
120	229	1.34±0.54[c]	5.10±0.52[C]	282.6±597.3
180	228	1.62±0.74[b]	5.45±0.44[B]	487.1±749.4
210	115	1.85±0.74[a]	5.62±0.36[A]	703.5±1022.3
Quarter position				
Front	404	1.45±0.65[a]	5.25±0.48[B]	342.4±635.8
Rear	398	1.39±0.64[b]	5.29±0.51[A]	416±749

[1]TEH = Teat-end hyperkeratosis; [2]LnqSCC = Log transformed quarter somatic cell counts; [3]NA = Not applicable; For each variable, values in columns with different superscript differ significantly (P<0.05)

Table 2

Results of binary logistic regression analysis depicting association of subclinical mastitis with variables under study

Source of variation	Z	P-value	Odds Ratio	95% Confidence Interval	
				Lower	Upper
[1]TEH score					
Normal	Reference				
Smooth	0.67	0.506	1.16	0.76	1.77
Rough to severely rough	3.61	0.000	5.77	2.23	14.95
Stage of lactation (day)					
30	Reference				
60	-0.39	0.698	0.88	0.47	1.67
120	1.10	0.272	1.35	0.79	2.31
180	6.35	0.000	5.84	3.39	10.06
210	8.08	0.000	52.26	20.02	136.42
Quarter position					
Front	Reference				
Rear	1.16	0.245	1.22	0.87	1.70

[1]TEH = Teat-end hyperkeratosis

(2001) also reported negative relationship between higher TEH scores and udder health. In contract to our results and those reported above, Zoche-Golob *et al.* (2015) found no significant relationship between TEH and SCM using milk SCC as an indicator. Teats with poor TEH scores have a thick and rough ring around teat orifice (Mein *et al.*, 2001) which affects teat-end's tight closure and, therefore, facilitate the entry of microorganisms into the teat canal. Moreover, the rough ring has cracks which bacteria can enter (Neijenhuis *et al.*, 2001) and evade the action of teat disinfectants.

The SCS also followed a non-linear curve over the lactation; the mean values 1st decreased up to 120 days during the lactation and then started increasing at the end of the lactation. With respect to quarter position, a significant difference ($P<0.05$) was noted in the levels of SCS (values being 5.25±0.48 and 5.29±0.51 for front and rearquarters, respectively). Such differences over the lactation, taking teat-end conditions into consideration, suggest the importance of a careful evaluation of milking machine related factors in order to prevent consequence of persistent poor teat-ends. Although front quarters had higher TEH scores than the rear ones, but SCC was found to be significantly higher in hind quarters which probably be due to the fact that hind quarters are placed closer to the ground and thus more prone to injuries.

As compared to quarters with normal and smooth teat-ends (TEH score =1+2), the risk for occurrence of SCM was significantly higher in quarters with rough to severely rough teat-ends (TEH score =3+4) (OR: 5.77; CI: 2.23-14.95; $P<0.001$) (Table 2). Further, as the lactation advanced, more proportion of quarters get infected with SCM; a significant difference occurred at 180th day (OR: 5.84; CI: 3.39-10.06; $P<0.001$) and 210th day (OR: 52.26; CI: 20.02-136.42; $P<0.001$) when compared with 30th day of lactation. Similar findings were also reported by Singh *et al.* (2013). Teats with a smooth and thin callosity ring possess lower risk of mastitis. On the other hand, teats with thick and rough callosity had highest incidence of mastitis (Neijenhuis *et al.*, 2001). Teats with poor TEH score have damaged teat orifices which might not close properly after the milking, and thus, leads to bacterial penetration comparatively easier than teats with normal TEH score. The present findings are in accordance with the observations of Bartlett *et al.* (1990). In contrast to SCC which differs significantly between front and rear quarters as discussed earlier, we could not establish any association between SCM and quarter position in this study.

Overall, the present study indicates that the severity of TEH increases as the lactation advances with the front teats being more affected as compared with the rear teats, and the quarters with high TEH score poses a potential risk for poor udder health in Holstein Friesian × Sahiwal crossbred dairy cows. Therefore, it may be recommended that teats should be observed for TEH at regular intervals (especially 3rd month of lactation onwards) with regard to evaluation of mastitis risk, milking procedures, milking machine characteristics and management practices at the dairy farm.

REFERENCES

Bartlett, C.P., Miller, Y.G., Anderson, R.C. and Krik, H.J. (1990). Milk production and somatic cell count in Michigan dairy herds. *J. Dairy Sci.* **73**: 2794-2800.

Besier, J., Lind, O. and Bruckmaier, R.M. (2016). Dynamics of teat-end vacuum during machine milking: types, causes and impacts on teat condition and udder health - a literature review. *J. Appl. Anim. Res.* **44**: 263-272.

Hillerton, J.E., Middleton, N. and Shearn, M.F.H. (2001). Evaluation of bovine teat condition in commercial dairy herds: 5 A portfolio of teat conditions. In: Proceedings of the 2nd International Symposium on Mastitis and Milk Quality, NMC/AABP, 13-15 September, Vancouver, BC, Canada. pp. 472-473.

Hogan, J., Gonzales, R., Harmon, R., Nickerson, S., Oliver, S., Pankey, J. and Smith, K. (1999). Laboratory Handbook on Bovine Mastitis. National Mastitis Council, Madison, WI.

Mein, G.A., Neijenhuis, F., Morgan, W.F., Reinemann, D.J., Hilleron, J.E., Baines, E.R., Ohnstard, I., Rasmussen, M.D., Timms, L., Britt, J.S., Farnsworth, R., Cook, N. and Hemling, T. (2001). Evaluation of bovine teat conditions in commercial dairy herds: 1. Non-infectious factors. In: Proceedings of the 2nd International Symposium on Mastitis and Milk Quality, NMC/AABP, 13-15 September, Vancouver, BC, Canada. pp. 347-351.

Mitev, J.E., Gergovska, Z.I. and Miteva, M.T. (2012). Effect of teat end hyperkeratosis on milk somatic cell counts in Bulgarian Black and White dairy cattle. *Bulg. J. Agric. Sci.* **18**: 451-454.

Neijenhuis, F., Barkema, H.W., Hogeveen, H. and Noordhuizen, J.P. (2001). Relationship between teat-end callosity and occurrence of clinical mastitis. *J. Dairy Sci.* **84**: 2664-2672.

Neijenhuis, F., Barkema, H.W., Hogeveen, H. and Noordhuizen, J.P.T.M. (2000): Classification and longitudinal examination of callused teat ends in dairy cows. *J. Dairy Sci.* **83**: 2795-2804.

Pantoja, J.C.F., Correia, L.B.N., Rossi, R.S. and Latosinski, G.S. (2020). Association between teat-end hyperkeratosis and mastitis in dairy cows: A systematic review. *J. Dairy Sci.* **103**: 1843-1855.

Rysanek, D., Olejník, P. and Babák, V. (2001). Vacuum fluctuation in short milk tube during peak milk flow. In ICAR Technical Series 7, Rosati, A., Mihina, S., Mosconi, C. (Edts.). pp.125- 130

Shearn, M.F.H. and Hillerton, J.E. (1996). Hyperkeratosis of the teat duct orifice in the dairy cow. *J. Dairy Res.* **63**: 525-532.

Singh, R.S., Bansal, B.K., Gupta, D.K., Randhawa, S.S. and Dhaliwal, P.S. (2013). The relationship of teat end hyperkeratosis with teat morphology and subclinical mastitis in Holstein Friesian × Sahiwal crossbred dairy cows. In: Proceedings of the 15th International Conference on Production Diseases in farm animals, 24-28 June, Uppsala, Sweden. p. 227.

Sterret, A.E., Wood, C.L., Mcquerry, K.J. and Bewley, J.M. (2012). Changes in teat-end hyperkeratosis after installation of an individual quarter pulsation milking system. *J. Dairy Sci.* **96**: 4041-4046.

Zoche-Golob, V., Haverkamp, H. Paduch, J.H., Klocke, D., Zinke, C., Hoedemaker, M., Heuwieser, W. and Krömker, V. (2015). Longitudinal study of the effects of teat condition on the risk of new intramammary infections in dairy cows. *J. Dairy Sci.* **98**: 910–917.

Zucali, M., Bava, L., Sandrucci, A., Tamburini, A., Piccinini, R., Daprà, V., Tonni, M. and Zecconi, A. (2009). Milk flow pattern,somatic cell count and teat apex score in primiparous dairy cows at the beginning of lactation. *Italian J. Anim. Sci.* **8**: 103-111.