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Effect of TEAT PROTECT spray and potassium permanganate teat dip in curing subclinical mastitis in crossbred cows of Villupuram district of Tamilnadu

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Abstract
A study was undertaken to assess the incidence of subclinical mastitis in crossbred cows of Villupuram district by using direct microscopic Somatic cell count (SCC). For this purpose sixty eight crossbred cows positive for subclinical mastitis (SCM) as per IDF (International Dairy Federation) criteria were randomly allotted in to two treatment groups. The cows in T1 group were subjected to post milking teat dip with one per cent Potassium permanganate ($KMnO_4$) solution immediately after milking. The cows in T2 group were sprayed with TEAT PROTECT sprays. Sixteen healthy cows were kept as control group and udder was washed with plain water. The treatments were carried out for 30 days. Udder health status was determined by recording the parameters viz. somatic cell count (SCC), average daily milk yield, udder colony count and teat colony count. The results revealed that TEAT PROTECT spray is superior to Potassium permanganate in curing SCM of crossbred cows.

Keywords: $KMnO_4$ teat dip, Somatic cell count, Subclinical mastitis, TEAT PROTECT spray

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INTRODUCTION

Mastitis is a major and perpetual problem of the dairy sector due to failure to follow basic management practices under field conditions incurring heavy financial losses as a result of reduction in milk yield, cost of treatment, loss due to discarded milk and loss of germ plasm due to culling of superior animals. The economic losses due to subclinical mastitis were estimated in the range of INR 21,677 to INR 88,340 per lactation (Rathod *et al.,* 2017). Subclinical mastitis in cattle and buffaloes is estimated to result in a loss of Rs.1592.87 and Rs. 892.42 per lactation, respectively (Sinha et al., 2014).

The subclinical form of the disease is 15 to 40 times more prevalent than its clinical counterpart, difficult to diagnose and usually persist longer in the herd causing production losses. Biswaadeep *et al*. (2015) carried out a cross sectional study to determine the prevalence of bovine subclinical mastitis in Jaipur district of Rajasthan. They recorded a high prevalence of subclinical mastitis in animal level, 67.27% and 74.55% by CMT and SCC respectively. They attributed this high prevalence to improper sanitation of environment, udder and milker´s hand.

Rathod *et al*. (2017) investigated the incidence rate of subclinical mastitis in Bidar, Karnataka. They have observed that poor hygiene of milkman in terms of health condition, zoonotic diseases, clean clothing, and proper hand washing before and after milking with antiseptic solution, also caused SCM in the dairy animals. The crossbred cows which contribute 85.2 per cent of the total milk production of Tamil Nadu are more vulnerable for mastitis because of stress due to higher productivity, inadequate feeding and tropical climate. Hence a study was conducted to compare the efficacy of a novel product (TEAT PROTECT spray) and one per cent Potassium permanganate ($KMnO_4$) teat dip in curing SCM in crossbred cows

MATERIALS AND METHODS

One hundred apparently healthy cows of Villupuram district were selected by multistage random sampling technique. The milk samples were screened by SCC method and interpretations was done as per Sing (2015). A cow with at least one quarter affected at the time of examination was considered positive for subclinical mastitis. The sixty eight crossbred cows positive for subclinical mastitis (SCM) as per IDF criteria and were randomly allotted in to two treatment groups. The cows in T1 group were subjected to post milking teat dip with one per cent Potassium permanganate ($KMnO_4$) solution immediately after milking. The cows in T2 group were sprayed with TEAT PROTECT sprays. Sixteen healthy cows were kept as control group and udder was washed with plain water. The treatments were carried out for 30 days.

Potassium permanganate Post teat dipping protocol: The cows in T1 group were provided post milking teat dip with one per cent Potassium Permanganate solution immediately before and after milking. The udder and teat of the animals were first washed with clean water and dried with clean cloth. Immediately after milking, nearly the whole external surface of the teat was dipped into dipping solution (one per cent Potassium Permanganate) which was left for about 30 seconds and teat was then dried by another clean cloth. The farmers were trained and requested to adopt the dipping practice for 30 days. After 30 days, milk samples were collected from animals and subjected to direct microscopic Somatic Cell Count and the values were recorded and compared with values of day 0.

Teat Protect spray protocol: In T2 group of cows the udder and teat of the animals were washed with clean water and dried with clean cloth, and the Teat-protect spray was applied onto the udder after milking. The gel was washed off just prior to subsequent milking. The farmers were trained and requested to adopt the practice for 30 days. After 30 days, milk samples were collected from animals and subjected to direct microscopic Somatic Cell Count and the values recorded and compared with values of day 0.

Control protocol: The udders of control animals were washed with plain water and dried with clean cloth for 30 days and milk samples were collected from animals and subjected to direct microscopic Somatic Cell Count and the values recorded and compared with values of day 0.

Milk collection procedure: Milk sample collection was carried out as per Biswaadeep *et al.,* (2015). At first quarters were washed thoroughly with lukewarm water and dried. The teat end of the quarter was swabbed with cotton soaked with 70% alcohol. The first 3-4 streams of milk were discarded and milk samples were taken from each quarter prior to the milking in separate clean containers. The containers were marked as right fore (RF), right hind (RH), left fore (LF) and left hind (LH) and collection was done first from near side and then from off side to avoid contamination of teat apices

Collection of teat and udder swab: The collection of udder and teat swabs was done as per Hubaety *et al.,* (2013). Sterile cotton swabs manufactured by HIMEDIA and supplied by TRPVB were used for sampling. Swabs were streaked on five locations of an area of 16 cm2 and placed in test tubes containing peptone saline, and transferred to the TRPVB laboratory (Centre for Animal Health Studies), TANUVAS in a cool box immediately for enumeration of CFU/swab. Colony counting of udder and teat swabs was performed on day 0 and day 30 of the trial as per USDA APHIS standard plate count protocol.

Collection of data of milk yield: The daily average milk yield in liters on day 0 and day 30 in the two treatment groups and control group were recorded

Direct microscopic counting of SCC: The estimation of somatic cell count (SCC) was carried out as per Singh(2015) involving the following steps.

Preparation of milk smears: Milk sample was thoroughly mixed, so as to obtain uniform distribution of cells. Ten micro liter of milk (0.01ml) from each quarter was spread over 1 cm² marked area on a grease free glass slide with the help of a platinum loop. The smears on the slides were left undisturbed at room temperature, air dried and preserved till staining was done.

Staining: The smear was fixed in Xylol for 5 minutes and stained with modified Lempert Newman stain by immersing in the stain for 20 seconds. Excess of stain was drained off and slides were air-dried. Then the slides were rinsed two or three times in water, drained, and rapidly air-dried, by gentle blotting with a filter paper. The smears were stained deep blue**.**

Calculation of working factor (WF) of the microscope: A binocular microscope was used with 10x oculars and 1.8 mm oil immersion objective. The diameter of the field was measured with the help of a stage micrometer.

Diameter of microscopic field = 0.16 mm or 0.016 cm

Area of the field = πr2 = 3.14 x (0.008)2 = 0.0002 sq.cm

Since 0.01 ml of milk was spared in 1.0 cm2, the possible number of fields counted in 1 sq. cm is 5000.

Milk volume represented by each field = 1/5000 x 1/100 = 1/500000 ml.

Hence, microscopic factor (MF) = 5, 00,000

Table 1. Effect of hygienic practices on the incidence of Subclinical mastitis in Villupuram district based on Somatic cell count, Udder and teat colony counts and milk yield in different groups.

Parameters	Days of Trial	$KMnO_4$ teat dip (T-1)		t-Value	TEAT PROTECT teat Spray (T-2)		t-value	Control (Water wash)		t-value
		0	30		0	30		0	30	
SCC- RF ($\times10^5$)	No. of Somatic cells ($\times10^5$) ±SD	3.67±0.232	1.14±0.239	12.415**	3.22± 0.291	2.52±0.149	3.023**	2.67±0.138	3.94±0.352	1.872NS
SCC-RH ($\times10^5$)		5.80±0.361	3.57±0.225	4.853**	6.01± 0.359	3.61±0.199	5.336**	2.66±0.152	4.37±0.474	1.624NS
SCC-LF ($\times10^5$)		4.09±0.248	3.33±0.191	3.009**	4.11± 0.241	2.92±0.177	4.483**	2.45±0.153	4.16±0.413	2.248*
SCC-LH ($\times10^5$)		9.77±0.404	6.44±0.293	4.043**	9.01± 0.348	4.54±0.274	6.330**	3.02±0.160	5.92±0.542	2.238*
Udder colony count- Log 10 Cfu /ml	Log 10 Cfu/ml ± SD	4.987±0.080	2.825±0.060	21.362**	4.97± 0.070	2.87±0.461	26.331**	4.76±0.153	4.97±0.302	3.240**
Teat colony count- Log 10 Cfu /ml		4.97±0.073	2.776±0.058	23.864**	4.968± 0.098	2.775±0.571	23.986**	4.86±0.086	4.98±0.049	5.713**
Milk yield(lit/day)	m. y. in lit ± SD	10.38±1.859	10.41±1.909	1.000NS	10.76± 1.986	11.35±2.013	6.866**	10.75±1.807	9.25±1.693	2.777*

mCMT: Modified California mastitis test; SCC: Somatic cell count.; RF: Right front quarter; RH: Right hind quarter; LF: Left front quarter; LH: Left hind quarter ;** Highly significant at one per cent level (P < 0.01); *Significant at five per cent level (P < 0.05) ; NS: Not significant (P>0.05)

Working factor (WF) = MF/ No. of fields counted i.e. 500000/25 = 20000

No. of cells per ml = Total no. of cells counted in 25 fields x WF (20000)

Cell counting: Cell counting was made by examining the smears under oil immersion objective as per the procedure used by Singh(2015). The cells were counted in 25 fields. Total number of cells counted was multiplied with the working factor to obtain the number of cells per ml of milk

Interpretation of counting results: Sample showing somatic cell count more than 5, 00, 000 cells is considered as positive case for subclinical mastitis as per the criteria cited by International Dairy Federation (IDF) and Hegde *et al.,* (2013).

Statistical analysis: The SCC values, teat swab and udder swab colony counts and daily average milk yield before and after treatment as observed in treatment and control groups analyzed by applying paired 't' test using IBM® SPSS® 23 of windows.

After solidification of agar, the plates were incubated in inverted position at 37°C for 24 hours. After incubation, bacterial cells grow into distinct colonies, which were counted with colony counter.

RESULTS AND DISCUSSION

The results of paired 't' test are presented in Table -1. In the $KMnO_4$ group, the mean ± SD of SCC values ($\times10^5$ cells/ml) of the right fore, right hind, left fore and left hind quarters of crossbred cows showed a significant (P<0.01) reduction from 3.67±0.232 to 1.14±0.239, 5.80±0.361 to 3.57±0.225, 4.09±0.248 to 3.33±0.191 and 9.77±0.404 to 6.44±0.293 respectively during the trial period. The mean ±SD of the udder and teat colony count (log 10cfu/ml) also showed a significant reduction (P<0.01) from 4.987±0.080 to 2.825±0.060 and from 4.97±0.073 to 2.776±0.058 during the trial period. The mean ±SD of the average daily milk yield (liters) also showed an increase from 10.38±1.859 to 10.41±1.909. This was in agreement with the observations of Hubaety *et al*. (2013) and Yasothai (2017) who envisaged positive results upon the usage of pre and post milking dip in cross bred dairy cattle. In contrary to these reports, Abinaya and Thangarasu (2017) have observed that suggested that one per cent of potassium permanganate solution is clinically not effective to control mastitis in dairy cows (Table-1).

In the TEAT PROTECT group, the mean ± SD of SCC values ($\times10^5$ cells/ml) of the right fore, right hind, left fore and left hind quarters showed a significant (P<0.01) reduction from 3.22±0.291 to 2.52±0.149, 6.01±0.351 to 3.61±0.199, 4.11±0.241 to 2.92±0.177 and 9.01±0.348 to 4.54±0.274 respectively during the trial period. The mean ±SD of the udder and teat colony count (log 10cfu/ml) also showed a significant reduction

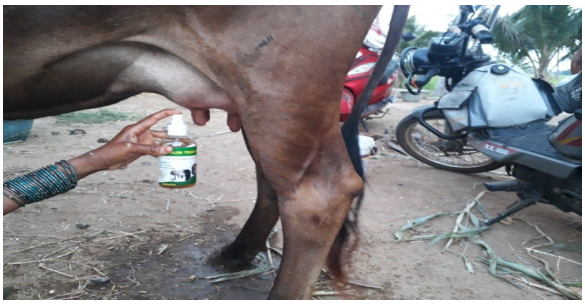

Fig. 1. *Application of TEAT PROTECT spray on the teats of cow.*

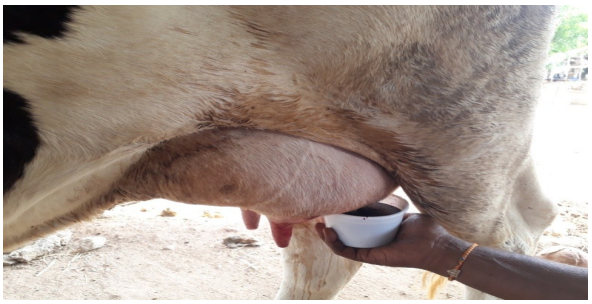

Fig. 2. *Application of $KMnO_4$ teat dip to the teats of cow.*

Table 2. Quarter cure rate (per cent) in different treatment and control groups of crossbred cows by screening with SCC in Villupuram district of Tamil Nadu and its analysis.

Result	T-1: $KmnO_4$ teat dip				T-2: TEAT PROTECT spray				Control (Water wash)			
	Days of trial		Quarter Cure per cent	Chi-square value	Days of trial		Quarter Cure per cent	Chi-square value	Days of trial		Quarter Cure per cent	Chi-square value
	0	30			0	30			0	30		
Positive	57	30	27 (47.36)	12.52**	60	12	48 (80.00)	43.52**	0	16	Nil (0.00)	18.29**
Negative	79	106			76	124			68	48		

(P<0.01) from 4.97±0.070 to 2.87±0.461 and from 4.968±0.098 to 2.775±0.571 during the trial period. The mean ±SD of the average daily milk yield (liters) also showed a significant (P<0.01) increase from 10.76±1.986 to 11.35±0.2.013. This was in agreement with the observations of Adarsh *et al.,* (2015) and Krishnakumar *et al.* (2017) studied the effect of herbal spray (AV/AMS/15), by treating 10 cows positive for SCM and observed that the average SCC values got reduction of 24.6 per cent of the cell count.

In the control group, the mean ± SD of SCC values ($\times 10^5$ cells/ml) of the right fore, right hind, left fore and left hind quarters showed an increase from 2.67±0.138 to 3.94±0.352, 2.66±0.152 to 4.37±0.474, a significant increase (P<0.05) from 2.45±0.153 to 4.16±0.413 and significant increase (P<0.05) from 3.02±0.160 to 5.92±0.542 respectively during the trial period. The mean ±SD of the udder and teat colony count (log 10cfu/ml) also showed a significant (P<0.01) increase from 4.76±0.153 to 4.97±0.302 and 4.86±0.086 to 4.98±0.049 during the trial period. The mean ±SD of the average daily milk yield (liters) showed a significant (P<0.05) reduction from 10.75±1.807 to 9.25±1.693.

The results showed that the application of teat protect spray for 30 days was highly effective in curing SCM as there was a significant (P<0.01) reduction in mCMT score and TANUCHEK score as well as, the SCC values in all the four quarters were significantly (P<0.01) brought below the cut-off levels fixed by IDF. But, application of 1 per cent $KMnO_4$ post milking teat dip for 30 days cured three quarters, as left hind quarters had SCC values above the IDF cut-off levels. The use of 1 per cent $KMnO_4$ post milking teat dip for another 30 days might cure all four quarters and further research is required in this context. In the control group, there was significant reduction of daily average milk yield, increase in the SCC values of all four quarters and significant increase in udder and teat colony count.

The results of quarter cure rates are presented in Table-2. In cows treated with $KmnO_4$, (T-1) a significant quarter cure per cent (P<0.01) was observed (47.36 per cent) as screened by SCC methods. (Table- 2) A significant (P<0.01) cure rate of 80.00 per cent was observed in cows treated with TEAT protect spray (T-2) as screened by SCC method and the TEAT protect spray was found superior to $KMnO_4$ in curing quarters of crossbred cows affected by SCM. There was a significant (P<0.01) incidence of new SCM infections that were observed in control group of cows which were negative for SCM at the beginning of the trial.

Conclusion

The TEAT protect spray was found superior to $KMnO_4$ in curing quarters of crossbred cows affected by SCM. There was a significant (P<0.01) incidence of new SCM infections that were observed in control group of cows. Regular application of a trusted germicidal spray like TEAT PROTECT is highly recommended to keep the levels of SCC below the cut-off point prescribed by the IDF and to increase milk production.

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