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

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 crossbreds 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 (KMnO₄) 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 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 Hubs et al., (2013). Sterile cotton swabs manufactured by HIMEDIA and supplied by TRPV 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 TRPV 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>TEAT PROTECT</th>
<th>t-value</th>
<th>t-value</th>
<th>t-value</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC - RF (×10⁵)</td>
<td>3.67±0.232</td>
<td>2.52±0.149</td>
<td>3.023²</td>
<td>1.872²</td>
<td>1.624²</td>
<td>2.248³</td>
</tr>
<tr>
<td>SCC - RH (×10⁵)</td>
<td>5.80±0.361</td>
<td>3.61±0.199</td>
<td>5.336⁴</td>
<td>2.66±0.152</td>
<td>4.37±0.474</td>
<td>4.16±0.413</td>
</tr>
<tr>
<td>SCC - LF (×10⁵)</td>
<td>6.01±0.351</td>
<td>4.19±0.177</td>
<td>4.43²</td>
<td>2.45±0.153</td>
<td>4.15±0.043</td>
<td>2.238³</td>
</tr>
<tr>
<td>SCC-LH (×10⁵)</td>
<td>9.77±0.404</td>
<td>4.97±0.274</td>
<td>6.33⁴</td>
<td>2.02±0.160</td>
<td>4.57±0.392</td>
<td>2.248³</td>
</tr>
<tr>
<td>Udder colony count (log 10 Cfu/ml)</td>
<td>2.87±0.461</td>
<td>2.67±0.138</td>
<td>26.33⁴</td>
<td>2.875±0.060</td>
<td>4.57±0.392</td>
<td>2.248³</td>
</tr>
<tr>
<td>Teat colony count (log 10 Cfu/ml)</td>
<td>4.977±0.571</td>
<td>4.97±0.073</td>
<td>23.96⁴</td>
<td>4.86±0.086</td>
<td>4.97±0.049</td>
<td>2.248³</td>
</tr>
<tr>
<td>Milk yield (l/day)</td>
<td>10.38±1.859</td>
<td>9.77±0.404</td>
<td>11.33²</td>
<td>10.75±1.807</td>
<td>9.25±1.693</td>
<td>2.777⁴</td>
</tr>
</tbody>
</table>

Working factor (WF) = WF/ 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)

**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)

RESULTS AND DISCUSSION

The results of paired ‘t’ test are presented in Table 1. In the KMnO₄ group, the mean ± SD of SCC values (×10⁵ 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, 6.01±0.351 to 3.61±0.199 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 10.36±1.859 to 10.41±1.909. This was in agreement with the observations of Hubaety et al. (2013) and Yasoithai (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 (×10⁵ cells/ml) of the right fore, right hind, left fore and left hind quarters showed a significant (P<0.01) reduction from 3.22±0.221 to 2.52±0.149, 4.96±0.241 to 2.92±0.177 and 9.01±0.348 to 4.968±0.241 respectively during the trial period.

The mean ±SD of the udder and teat colony count (log 10cfu/ml) also showed a significant reduction...
The results of quarter cure rates are presented in Table 2. In cows treated with K\text{mnO}_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 K\text{mnO}_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 K\text{mnO}_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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