**SUMMARY**

**Introduction** - Teat sealing is one preventive tool for mastitis control to prevent the access of the bacteria into the mammary gland. Intramammary teat sealants (ITS) have a high potential as preventive management among animal which do not have history of mastitis.

**Aim** - The general aim of the study was to evaluate the intramammary teat sealant (ITS) for the prevention of subclinical mastitis (SCM). It also aimed to compare the Somatic Cell Count (SCC) and California Mastitis Test (CMT) values of buffaloes infused with ITS device to those buffaloes without ITS, response of primiparous and multiparous buffaloes and determine the adverse reaction based on the changes on teat size and behavior of the treated animals.

**Materials and methods** - Eleven purebreed and healthy female buffaloes were enrolled in the study. Buffaloes were infused with ITS one month before the expected calving date. Milk collection was done every week for one month.

**Result and discussion** - Result showed that there was significant increase on the SCC of buffaloes infused with ITS in comparison to buffaloes without ITS based on SCC and CMT. Higher percentage occurrence of SCM was observed among buffaloes sealed with ITS in comparison to unseal. No significant difference was observed between treated multiparous and primiparous buffaloes. It showed that there was no significant difference on the size of the teat after 4-6 hours and 24 hours post infusion. Changes of behavior upon infusion of ITS were observed.

**Conclusion** - The use of ITS as preventive management in the occurrence of SCM should be carefully employed in lactating buffaloes. Since this product is already available in the market, adjunct use of antiseptic and proper infusion should be observed. The concept of reducing bacterial contaminants thru the teat via teat sealing is a rational one but based on the study it is not an absolute protection to the lactating animals particularly on water buffaloes maybe due to anatomical difference compare to cattle where this ITS is patterned. Employment of proper management such as regular cleaning and disinfection of the pens is still the best way of controlling mastitis in water buffaloes.

**KEY WORDS**

Internal Teat Sealant, Somatic Cell Count, California Mastitis Test.

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**INTRODUCTION**

Mastitis is the inflammation of mammary glands associated with physical and microbiological changes. This is one of the most common diseases in dairy herd. Mastitis may be described as peracute, acute, sub-acute or chronic according to its severity and duration. It may also be clinical or subclinical. Clinical mastitis shows evidence of inflammation such as pain, heat, swelling of affected quarter of gland and abnormality of milk as either clots or flakes. Subclinical mastitis (SCM) does not show signs of inflammation but milk production decreases and commonly detected by changes in milk composition. However, milk color and smell may not differ from milk produced by non-mastitic animal. Economical losses are due to loss in milk production, discarding abnormal milk and milk withheld from cows treated with antibiotics, degraded milk quality and decrease price of milk. Consequently, cost of production can increase due to costs of drugs, veterinary services, diagnostic fees and increased lab costs. Related losses are due to increased risk of subsequent mastitis, herd replacement, and problems related to antibiotics residues in milk. Common farmers are not very familiar with diagnostic techniques for monitoring and detecting subclinical infection. Diagnosis of SCM can be done by California Mastitis Test (CMT) and Somatic Cell Count (SCC). Some of the preventive measures such as use of antiseptic, regular cleaning of the surroundings, dipping, may not be as effective as expected. Animals during its dry period are exposed to pathogen causing increase incidence of mastitis in the herd. Treating animal with antibiotic intramammary infusion usually increases antibiotic residue in milk and it can be a public health concern. One preventive tool for mastitis is the sealing of the mammary gland to prevent the access of the bacteria into the mammary gland prior to lactation. The apparent advantage...
of this preventive technique is that withdrawal period of antibiotic used in treating mastitis are no longer necessary. Antibiotic residue in milk may not be an issue and milk is fit for human consumption. Hence, this study will evaluate the potential of ITS in preventing subclinical mastitis in buffaloes.

MATERIALS AND METHODS

Identification of experimental animals

Eleven female Murrah buffaloes were enrolled in the study. These buffaloes have functional teats, non-lactating pregnant about to calve after one month, and no history of SCM. The treatments were as follows: three primiparous and three multiparous buffaloes were given teat sealant, while three primiparous and two multiparous buffaloes were not given teat sealant.

Animal management

Buffaloes enrolled in the study were kept together with other dry buffalo cows in one barn. The animals were transferred to the maternity house one month before the expected calving date. About 30 to 40 heads were within the same pen. The animals enrolled were identified through their individual ear tag number. Rationing of hay and feeds was done. Cleaning of pen was done twice a day.

Administration of ITS

Six dried buffaloes were infused with ITS 30±5 days before the expected calving date. Before the infusion, individual quarters were washed with clean water and soap. Teat end was scrubbed with 70% alcohol. The mammary gland was dipped in 10% iodine solution for 15 seconds to disinfect. The far teats were disinfected first before the near teats, to avoid accidental contamination of previously disinfected teats. The ITS was infused in each teat or quarter of the animal. This was done by inserting the nozzle into the teat, grasping the base of the teat near the udder attachment with two fingers pressed firmly together and applying gentle continuous pressure to the plunger until the paste is completely injected. One syringe with 4 mg of sealant per quarter was used. Pressure was avoided after the infusion to let the seal remain in the teat canal. Teats were dipped again in 10% iodine solution for 15 seconds.

Pre-infusion, the animal was observed for normal behavior and the circumference of the teat mid-part was measured. After four to six hours and 24 hours post-infusion, the animal was observed again for any unusual behavior such as scratching, restlessness and nervous signs.

Post-calving management

Immediately after calving, ITS was removed by manual stripping of teat. This was done by grabbing the top of the teat where it meets the udder-and working all the way down. The entire quarter was stripped by starting at the top and working all the way down. Milk were collected on the first, 2nd, 3rd and 4th week-postcalving and tested for SCM using SCC and CMT.

A total of 50 ml of milk was collected per quarter or teats. Milk collected was placed in a collection tube until analysis. Automated somatic cell counter (Fossomatic Minor, Denmark) was used to get the SCC of each sample. Furthermore, no bacteriological test was performed since in a previous study, it had been established in a parallel bacteriological test using culture method and PCR assay a dominant presence of Staphylococcus species in milk with +1, +2 and +3 reading by CMT. In reference to this, no bacteriological test was further performed rather assumed that turning positive to CMT or SCC connotes reoccurrence of SCM.

Statistical analyses

Analysis of variance (ANOVA) was employed for the comparison of treatments using the SCC. Meanwhile, chi-square test was employed for the analysis of CMT results. For the comparison of effects with consideration to the parity number, the treatment and control categories were separated. Also, the study determined the difference in the percentage occurrence of SCM based on SCC and CMT test results among buffaloes infused with ITS compared to control animals using descriptive statistics. ANOVA was used to describe the post-infusion changes in the mammary glands of the treated buffaloes based on the measurement of mid-diameter of the teat. Descriptive statistics was also used to describe change in the behavior of the animal.

RESULTS AND DISCUSSION

The study determined the efficacy of ITS in preventing SCM in lactating primiparous and multiparous buffaloes based on SCC and CMT. Buffaloes with SCC of ≥200,000 cells/ml and CMT score of 1, 2, 3 are said to be positive to SCM.

Efficacy of ITS based on SCC

Results showed (Table 1) the mean comparison of SCC in buffaloes with (treatment) and without ITS (control). The mean SCC of the treatment is significantly higher (803.900 cells/ml) as compared to the control (301.200 cells/ml). Both SCC of the two groups of animals were significantly higher as compared to the published normal value of not beyond 200,000 cells.

The SCC result per teat per week was the basis of the percentage occurrence of SCM in observed buffaloes. Results showed that there was a higher percentage (84%) of subclinically mastitic teats in control animals as compared to treatment (79%) on the first week of lactation. On the 2nd week, percentage occurrence (26%) of mastitis in the control decreased while it increased in the treatment (88%). On the third week, both groups of animal had a reduced occurrence of mastitis however, treatment had higher (54%) cases as compared to the control (5%). On the 4th week, there was an increase in the occurrence of subclinically mastitic teats both in the treatment (58%) and control (11%), however, treated group showed a higher occurrence than the control.

Table 1 - Mean comparison of somatic cell count in buffaloes with and without internal teat sealant.

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Mean (x10³)</th>
<th>N (number of teats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>803.9*</td>
<td>88</td>
</tr>
<tr>
<td>Control</td>
<td>301.2*</td>
<td>76</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different.
ITS, according to Bradley et al.1 and Kromker et al.4, can reduce the number of bacteria in the teat as it seals the entry of organism. Laven and Lawrence5 showed the efficacy of using teat sealant in preventing occurrence of subclinical mastitis in cow, however, the present study has contrary outcome in buffaloes.

**Efficacy of ITS based on CMT**

Based on the CMT result, the study showed that there were significantly more subclinically mastitic teats in treated teats as compared to non-treated teats. The percentage occurrence of SCM based on CMT on weeks 1, 2, 3 and 4 were 79%, 63%, 33% and 33%, respectively, on treated animals. On the other hand, the control showed 84%, 26%, 21% and 0% percentage occurrence on weeks 1, 2, 3, and 4, respectively.

Based on the results of SCC and CMT tests to determine the occurrence of subclinical mastitis in buffaloes with and without ITS, there were more affected teats in treated animal as compared to the non-treated animal.

**Efficacy of ITS based on parity**

The comparison of mean SCC of multiparous and primiparous buffaloes was shown in Table 2. The mean SCC of multiparous buffaloes with and without ITS were 657.200 cells/ml and 285.300 cells/ml, respectively.

On the other hand, the mean SCC of primiparous buffaloes with and without ITS were 950.700 cells/ml and 328.500 cells/ml, respectively.

Statistical difference was only observed between the treated and the untreated primiparous buffaloes however, no significant difference was observed between multiparous and primiparous buffaloes with ITS.

The percentage occurrence of SCM in primiparous buffaloes in week 1 started low at 58%, which fluctuated on subsequent weeks. While the percentage occurrence of SCM in multiparous buffaloes was high on the 1st two weeks, it showed subsequent decreased in the succeeding weeks.

**Table 2 - Comparison of mean SCC of multiparous and primiparous buffaloes.**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Mean (x10³)</th>
<th>N (number of teats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiparous Control</td>
<td>657.2 ab</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>285.3 b</td>
<td>48</td>
</tr>
<tr>
<td>Primiparous Control</td>
<td>950.7 a</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>328.5 b</td>
<td>28</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different (P > 0.05).

**Table 3 - Comparison of mean circumference of the mid-part of the teat (cm).**

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Right front</th>
<th>Left front</th>
<th>Right hind</th>
<th>Left hind</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td>1st</td>
</tr>
<tr>
<td>Treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.6 a</td>
<td>8.8 a</td>
<td>9.3 a</td>
<td>8 a</td>
<td>8.5 a</td>
</tr>
<tr>
<td>Control</td>
<td>7.3 a</td>
<td>7.3 a</td>
<td>7.7 a</td>
<td>6.7 a</td>
</tr>
</tbody>
</table>

Note: Means within a row (a, b, c) having the same letter script are not significantly different from each other at 5% probability level by Analysis of Variance.

1st - before infusion.
2nd - 4-6 hours post-infusion.
3rd - 24 hours post-infusion.
CONCLUSION AND RECOMMENDATION

Based on this initial use of ITS in dry buffaloes, the device did not satisfactorily prevent the occurrence of SCM. The SCC and CMT results were more positive in ITS treated animal as compared to the non-treated. No significant protection was given to multiparous animals which are known to be more prone to SCM. Teat disinfection before application of ITS did not help also in the prevention of occurrence of SCM.

The study, however, showed that ITS are safe to the mammary glands as no significant changes in the size of mammary teats as well as behavior patterns were seen in infused buffaloes.

The use of ITS as preventive management in the occurrence of SCM should be carefully employed in lactating buffaloes. Since this product is already available in the market, further testing and observation should be employed to determine the case to case effect of the product to animals. Further bacteriological testing of teat should be done before and after the sealing.

Employment of proper management such as regular cleaning and disinfection of the pens is still recommended in controlling mastitis in water buffaloes.

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References