Comparison of Different Diagnostic Tests in Subclinical Mastitis in Dairy Cattle

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ARTICLE INFO

Received: August 08, 2014
Revised: August 16, 2014
Accepted: September 06, 2014

Key words:
Cattle
California mastitis test
Electrical conductivity
Somatic cell count
Subclinical mastitis

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ABSTRACT

The present study was undertaken to find out the comparative efficacy of commonly used diagnostic tests for detection of subclinical mastitis in dairy cattle of Andhra Pradesh. Present communication aims to find out the specificity, sensitivity and predictive value of different indirect tests California mastitis test (CMT), electrical conductivity (EC) and somatic cell count (SCC) taking cultural test as standard in subclinical mastitis affected cattle. Out of 135 quarter milk samples subjected to CMT, EC and SCC taking cultural examination as standard, the per cent accuracies were found to be 73.33, 70.37 and 71.00 respectively. The false positive reactions were more in CMT (24.60%) followed by SCC (23.70%) and EC (20.40%) whereas the false negative reactions were highest in EC (34.90%) followed by SCC (31.60%) and CMT (28.60%). The sensitivity, specificity and predictive value of different tests were studied and it was found that EC had the highest specificity (84.84%) and predictive value (79.59%) with lowest sensitivity (56.62%) than compare with the other diagnostic methods for diagnosis of subclinical mastitis in cattle. Electrical conductivity can be used as the decision criteria to treat or to cull the animals in herds with high prevalence of subclinical mastitis.

INTRODUCTION

Clinical mastitis is an individual problem which is characterized by the changes in the udder and milk drawn from it, whereas, sub clinical mastitis is herd problem because it constitutes a reservoir of infection which could be transmitted to other animals of herd. It has also been observed that the incidence and the patterns of causative agents markedly differ from place to place, herd to herd, and time to time. Diagnosis of mastitis at sub clinical stage is vital because changes in the udder tissue take place much earlier than they become apparent. SCM also goes unnoticed and remain a depot for spreading infection to the herd mates (Gera et al., 2006). Subclinical mastitis associated with no apparent changes in the udder or milk composition, although microorganism can be isolated by appropriate culture techniques. Cows with subclinical mastitis had decreased milk production by 10 to 20 per cent with undesirable effect on its constituents and nutritional value rendering it of low quality and unfit for processing. The diagnosis of subclinical mastitis (SCM) is problematic since the milk appears normal but usually has an elevated somatic cell count (Forsback et al., 2010). The invisible changes in subclinical mastitis can be recognized indirectly by several diagnostic methods including the California mastitis test (CMT), the Modified White Side test (MWT), Somatic cell count (SCC), pH, chlorine and catalase tests. These tests are preferred to be screening tests for subclinical mastitis as they can be used easily, yielding rapid as well as satisfied results (Leslie et al., 2002). The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the somatic cell count (SCC) and microbiological status of the quarter. Bacteriological culture of milk samples is the standard method for identifying mastitis. However, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted...
of etiological agents (bacteria). A loop full of milk samples were inoculated into nutrient broth and incubated at 37° C for 24 hours aerobically and then a loop full of broth culture were streaked on nutrient agar plates. Based on morphology and Gram’s staining properties, cultures were inoculated into specific/selective media like Mac Conkey, Mannitol salt agar, and Eosin-Methylene blue agar (Reddy et al., 2011; Reddy et al., 2014).

Percent sensitivity, specificity and percent accuracy were calculated by the formulae of Thrusfield (2005).

RESULTS

Various grades of CMT reaction obtained on screening of quarter milk samples by CMT. While 1154 quarter milk samples were negative for CMT reaction, 445 were culturally positive, further 247, 152, 46 quarter milk samples were showing, +, ++ and +++ CMT reaction respectively. The mean (+ SE) electrical conductivity of milk from infected quarters was 270±30 whereas that of uninfected quarters were 360±40 and there was a significant difference (P) between the mean electrical conductivity of culturally positive and negative quarters from the subclinical mastitis cows. Leukocyte count of 59 (44.06%) quarters belonging to animals showed a count of 3,00,000 cells and above per ml of milk.

A total of 135 quarter samples from 35 animals were examined with different tests and the results were compared. Out of the 135 milk samples screened, 65, 49, 59 and 69 samples were positive by CMT, SCC, EC, and cultural examination respectively (Table 1).

Out of these, 65 (48.14%) quarter samples from 29 animals were positive for CMT, where the incidence was 48.74% and 82.85% respectively quarter wise and animals wise. Similarly in electrical conductivity test, 49 (36.25%) quarters belonging to 19 (54.54%) animals showed an electrical conductivity of 270±30 indicating presence of subclinical mastitis. Somatic cell count showed 59 (44.06%) quarters belonging to animals 23 (65.71%) was suffering with SCM. The cultural examination of the same samples could yield the bacteria from 69 (51.11%) quarters of 28 (80%) animals as an evidence of mastitis infection.

The percent accuracy of various diagnostic tests for the detection of SCM, when cultural test was taken as standard and it was shown in Table 2 and Figure 4. The per cent accuracy of CMT, EC and SCC was 73.33, 70.37 and 71.00 per cent respectively. The false positive reactions were more in CMT (24.60%) followed by SCC (23.70%) and EC test (20.4%) and the false negative reactions were more in EC (34.90%) followed by SCC (31.60%) and CMT (28.60%).

Among these three indirect tests, the CMT was 75.38 per cent; EC was 79.59 per cent; whereas SCC was 76.27 percent true positive when compare with the cultural examination of the milk obtained from the individual cattle suffering with the subclinical mastitis.

The Table 3 & Figure 5 shows that the sensitivity and specificity followed by predictive value of positive test. Among the three tests, CMT (71.01%) had the highest sensitivity, followed by SCC (65.21%) and EC (56.52%). Highest specificity was notice with the EC (84.84%) followed by SCC (78.78%) and CMT (75.75%).
DISCUSSION

In SCM, irrespective of the etiological agents involved, the first pathological change noticed is the passage of leukocytes and erythrocytes into the milk as a result of increased permeability of udder capillaries to inflammatory reaction. The intensity of the inflammation can be estimated qualitatively by CMT and quantitatively by SCC. Similarly, the diffusion of sodium and chloride ions into the milk also increases which can be detected by EC. So the presence of the causative agent in the milk can be identified by cultural examination. Thus in the present study four different diagnostic tests namely, CMT, SCC, EC and cultural examination were used for the detection of SCM. The sensitivity and specificity of the above three tests were determined by considering cultural examination as standard test. The reasons for variation in the sensitivity and specificity of different diagnostic tests in the present study might be due to the fact that they are designed to detect different types of changes in the sub clinically infected milk.

Sharma et al. (2010) compared the sensitivity of the CMT, SLST, and SCC for the detection of SCM in dairy cows and stated that CMT was the most accurate, reliable, diagnostic method after cultural isolation and SCC under field conditions. Siji and Vijaykumar (2007) and Islam et al. (2010) reported the better performance of CMT in detecting SCM among the other indirect tests. Sharma et al. (2010) reported that the sensitivity of the CMT was 86.07 percent, specificity was 59.70 percent, accuracy was 75.52 percent, positive predictive value was 76.21 percent, and negative predictive value was 74.07 percent, and stated the CMT was the most accurate reliable diagnostic method.

In the present study the SCC of more than 3, 00,000/ml of milk was considered as positive for SCM. Sorana et al. (2010) reported that the positive diagnosis was

Table 1: Detection of subclinical mastitis using different diagnostic tests

<table>
<thead>
<tr>
<th>Name of test</th>
<th>Number of quarters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tested</td>
</tr>
<tr>
<td>CMT</td>
<td>135</td>
</tr>
<tr>
<td>EC</td>
<td>135</td>
</tr>
<tr>
<td>SCC</td>
<td>135</td>
</tr>
<tr>
<td>Cultural isolation</td>
<td>135</td>
</tr>
</tbody>
</table>

Fig. 1: California mastitis test paddle.

Fig. 2: Mastitis detector (Draminski).

Fig. 3: Somatic cells counter (DeLaval).

Fig. 4: Percent accuracy, false positive and false negative reactions of various diagnostic tests employed for diagnosis of SCM taking cultural test as standard.

Fig. 5: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural test as standard.
confirmed by the increased number of somatic cells present in milk. The values obtained were between 5, 00,000 and 1.5 million cells/ml in SCM and in healthy cows, somatic cell count has not exceeded the value of 2, 70,000 cells/ml. Sharma et al. (2010) reported the sensitivity of the SCC was 88.60 percent; specificity was 97.76 percent; accuracy was 91.94 percent; positive predictive value was 84.52 percent; negative predictive value was 98.33 percent and negative predictive value was 84.52 percent.

Among the indirect tests the measurement of EC of milk has an advantage over the mastitis detection procedures in that the result is made available immediately without additional effects and large number of animals can be screened by a single visit. EC is considered as sensitive and gives the result on the spot and can help to detect quarter wise prevalence and farmers can easily use this instrument to screen dairy animals for SCM (Muhammad et al., 2011).

Jorge et al. (2004) opined that EC is better than CMT, whereas Janzekovic et al. (2009) claimed that CMT and EC don’t exclude themselves mutually complementally to each other. Guven et al., (2012) concluded that EC showed similarity with the CMT and the SCC in the detection of SCM furthermore, its reliability would further increase when used together with the other diagnostic methods. Seguya and Mansell et al. (2010) compared the efficacy of EC by taking cultural test as a standard test. Gaspardy et al. (2012) reported that shortly after calving the EC value generally decreases; however, it was discovered that from the thirteenth week onwards, substantial differences arise between the mastitis and healthy groups of cows and observed a significant (P<0.001) increase in EC before the detection of clinical mastitis.

Although, the EC may have some practical advantages in comparison to other diagnostic methods, the predictive value was generally, poor. However, while judging the results of different diagnostic tests of SCM, one should consider the fact that the EC detects the chemical changes, CMT and SCC the cultural examination indicates the presence of bacteria. In essence the cultural examination of milk is very essential as the ultimate aim is detection and prevention of infection.

In the present study per cent accuracy of various (CMT, EC, SCC) tests taking cultural test as standard is summarized that electrical conductivity test has high specificity (84.84%) and predictive value (79.59%) with lowest sensitivity (56.52%) than compare with other diagnostic tests. Though the sensitivity is low, a high specificity and predictive value of the positive test in the present study enables EC to be used as the decision criteria to treat or to cull the animals in herds with high prevalence of sub clinical mastitis in dairy.

Acknowledgement

Corresponding author expressed his thankfulness to the authorities of the Sri Venkateswara Veterinary University, Tirupati for providing the facilities required for the completion of present study. Special thanks to the staff of College of Veterinary Science, Tirupati for their help during the study.

REFERENCES


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<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Total samples examined</th>
<th>Test positive samples</th>
<th>True positive</th>
<th>False positive</th>
<th>True negative</th>
<th>False negative</th>
<th>Percent Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT</td>
<td>135</td>
<td>65</td>
<td>75 (75.38%)</td>
<td>24 (24.60%)</td>
<td>76 (71.40%)</td>
<td>20 (28.60%)</td>
<td>73.33%</td>
</tr>
<tr>
<td>EC</td>
<td>135</td>
<td>49</td>
<td>59 (79.50%)</td>
<td>15 (20.40%)</td>
<td>85 (65.10%)</td>
<td>30 (34.90%)</td>
<td>70.37%</td>
</tr>
<tr>
<td>SCC</td>
<td>135</td>
<td>59</td>
<td>69 (76.27%)</td>
<td>21 (23.70%)</td>
<td>79 (68.40%)</td>
<td>24 (31.60%)</td>
<td>71.00%</td>
</tr>
<tr>
<td>Cultural isolation</td>
<td>135</td>
<td>69</td>
<td>108 (100%)</td>
<td>--</td>
<td>--</td>
<td>97 (100%)</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

(Figures in parenthesis indicates percent)

Table 2: Per cent accuracy of various diagnostic tests taking cultural test as standard

Table 3: Sensitivity, specificity and predictive value of different diagnostic tests taking cultural test as standard

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