DIAGNOSIS OF SUBCLINICAL MASTITIS: A COMPARATIVE STUDY OF DIFFERENT TESTS

Rajiv Singh*, B.K. Bansal, S.K. Uppal and D.S. Malik

Department of Veterinary Medicine
Punjab Agricultural University, Ludhiana - 141001, India

ABSTRACT

Detection of cases of subclinical mastitis presents difficulties because in early stages of the disease clear cut clinical changes are not manifested. A total of 200 milk samples from apparently healthy quarters of 50 healthy crossbred dairy cows in different stages of lactation, were taken and subjected to estimation of various constituents like lactose, Na+, K+ and Cl- alongwith indirect diagnostic tests like electrical conductivity, sodium lauryl sulphate and bromothymol blue card test. Each reading of different tests was compared with bacteriological finding and overall percentage of agreement was calculated for each test. The percentage of agreement of electrical conductivity, lactose, BTB, SLST, sodium, chloride and potassium was 69.38, 71.47, 77.66, 76.15, 67.63, 70.87 and 55.00 respectively.

INTRODUCTION

Mastitis continues to be a major problem concerning dairy industry. The disease manifests in various forms depending upon severity and duration of infection. It is sometimes clearly apparent but more often it is a hidden disease (subclinical mastitis). As such different methods have been devised from time to time which can be used to detect such cases but we need specific detection methods for early diagnosis and therapy. Culturing of milk samples for identifying the microbial pathogen is time consuming and costly. Moreover, the bacteria are not necessarily shed at each milking even when an infection is present. The present study was designed to know the efficacy of different tests for detection of subclinical mastitis in cows.

MATERIAL AND METHODS

A total of 200 milk samples from apparently healthy quarters of 50 healthy crossbred dairy cows in different stages of lactation, kept at an organised dairy farm were taken. After discarding first few streaks, about 12-15 ml of foremilk sample was collected from each quarter in individual sterilized test tubes taking all possible aseptic precautions. The milk samples were then immediately transported to laboratory and subjected to bacteriological examination (Collee et al., 1989). The apparently healthy quarters which showed bacterial growth were considered positive for subclinical mastitis (73 in our study).

Sodium lauryl sulphate test (SLS) and Bromothymol blue card test (BTB) were conducted and interpreted as per the standard method of Pandit and Mehta (1969) and Randhawa et al., (1983), respectively. The milk samples were further subjected to estimation of chloride content as per the standard volumetric procedure described in ICAR (1979). Estimation of lactose was done in milkscan. (Model

* Present Address : S.K. Univ. Agric. & Tech., R.S. Pura, Jammu-180001, India.
The milk samples were stored at -20°C till concentration of Na⁺ and K⁺ was assessed on flame photometer (Model CL 22D ELICO Pvt. Ltd. Hyderabad) as per the method of Lindsey and Norvell (1969).

The percentage of agreement of various tests was calculated by comparing each reading of different tests with bacteriological finding. If the positive reading of above said indirect tests coincided with bacteriological finding, then it was considered as true positive (TP). When milk sample was negative on indirect test as well as on bacteriological examination it was considered as true negative (TN). The milk samples which were +ve with indirect tests and negative on cultural findings were considered as false positive (FP) and vice versa as false negative (FN).

The percentage of agreement was calculated according to formula suggested by Ewbank (1962).

\[
\text{Percentage agreement} = \frac{1}{2} (\text{TP} \% + \text{TN} \%) - (\text{FP} \% + \text{FN}\%)
\]

RESULTS AND DISCUSSION

The relationship between different indirect tests and bacteriological examination is presented in Table 1.

Electrical conductivity: Variation in the conductivity level of milk from normal cows was observed, which made it difficult to establish consistent value for normal animal. To overcome this the conductivity ratio (CR) was calculated and it was observed that CR of 1.10 was optimal giving smallest number of FP and FN determinations. Greatrix et al. (1968) and Kitchen (1981) also observed that improvement in diagnosis could be obtained by simultaneously comparing the conductivity value of all the four quarters of the animal. The percentage of FP and FN samples was 9.38 and 11.03 respectively (Table 1). The results are in agreement with the findings of Duijs (1980) and Fernando et al. (1982), who reported 77 to 98 per cent efficacy of EC in diagnosing infected quarters.

Sodium, potassium and chloride: The increase in Na⁺ and Cl⁻, and decrease in the K⁺ levels had been used as methods for monitoring mastitic infection. The percentage of agreement for Na⁺, K⁺ and Cl⁻ estimations with bacteriological examination to diagnose subclinical cases at threshold value of 68 mg/dl, 124 mg/dl and 0.145 per cent, respectively was found to be 67.63, 55.00 and 70.87, respectively (Table 1). Thus the results of present investigation revealed greater reliability of Na⁺ and Cl⁻ to diagnose infected quarters as compared to K⁺.

Similarly, Fernando et al. (1985) observed that Na⁺, K⁺ and Cl⁻ at threshold values of 77 mg/dl, 126 mg/dl and 145 mg/dl, respectively gave 10.40, 38.60 and 15.50 per cent FP results, respectively. Fernandez et al. (1990) found that Na⁺ at threshold of 789 mg/l of quarter milk gave 7.34 per cent FN and 9.61 per cent FP results.

Lactose: The decrease in level of lactose has also been used as one of indirect method for diagnosing subclinically infected quarters. The threshold value of 4.80 gm/dl for distinguishing normal and infected quarters gave 88.73 per cent TP and 92.25 per cent TN results when compared with the bacteriological examination of milk samples. Renner (1974) using threshold value of 4.55 per cent for lactose reported 6 per cent FP and 6 per cent FN results, while Randhawa et al. (1983) reported that lactose estimation was 74.40 per cent efficacious in diagnosing subclinical mastitis.
Table 1. A comparative study of various indirect tests and bacteriological examination of milk samples in cows for diagnosing sub-clinical mastitis

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Name of test</th>
<th>Sample bacteriologically positive</th>
<th>Percentage agreement</th>
<th>True</th>
<th>False</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>True</td>
<td>False</td>
<td>Total</td>
</tr>
<tr>
<td>1.</td>
<td>Electrical conductivity</td>
<td>73</td>
<td>58</td>
<td>64</td>
<td>121</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(90.62)</td>
<td>(9.38)</td>
<td>(88.97)</td>
</tr>
<tr>
<td>2.</td>
<td>Lactose</td>
<td>73</td>
<td>63</td>
<td>71</td>
<td>119</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(88.73)</td>
<td>(11.27)</td>
<td>(92.25)</td>
</tr>
<tr>
<td>3.</td>
<td>BTB</td>
<td>73</td>
<td>62</td>
<td>68</td>
<td>124</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(91.01)</td>
<td>(8.83)</td>
<td>(93.94)</td>
</tr>
<tr>
<td>4.</td>
<td>SLST</td>
<td>73</td>
<td>59</td>
<td>64</td>
<td>125</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(92.19)</td>
<td>(7.81)</td>
<td>(91.91)</td>
</tr>
<tr>
<td>5.</td>
<td>Cl⁻</td>
<td>73</td>
<td>64</td>
<td>73</td>
<td>118</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(87.67)</td>
<td>(12.33)</td>
<td>(92.91)</td>
</tr>
<tr>
<td>6.</td>
<td>Na⁺</td>
<td>73</td>
<td>63</td>
<td>73</td>
<td>117</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(86.30)</td>
<td>(13.70)</td>
<td>(92.12)</td>
</tr>
<tr>
<td>7.</td>
<td>K⁺</td>
<td>73</td>
<td>50</td>
<td>58</td>
<td>119</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(86.20)</td>
<td>(13.80)</td>
<td>(83.80)</td>
</tr>
</tbody>
</table>

- Other indirect diagnostic tests: With SLS test the percentage of agreement with bacteriological examination in diagnosing subclinical infection of cows was found to be 76.15 per cent. These findings are corroborated by the results of Randhawa et al. (1983) and Kumar (1990). The percentage of agreement of BTB test with bacteriological examination was 77.66 for subclinically infected quarters of cows. However, Randhawa et al. (1983) reported a higher efficacy of 80.30 per cent.

The false positive results observed in the present study could be due to the fact that generally the shedding of bacteria from udder is not constant during milking or at different milkings and some pathogens might be missing from milk samples taken for study (Wesen et al., 1968). Moreover, these compositional changes like Na⁺, K⁺, Cl⁻, lactose, total leucocytic count etc. and the pH of milk can also be brought by other causative agents like fungi, virus and mycoplasma for which isolation was not tried in present study. In addition, it had been also observed that these compositional changes sometimes remained elevated above normal level even after bacteria were destroyed by defense mechanism or by self cure. The other possible reason for the false positive results could be the variation in the concentration of these milk constituents during the different stages of lactation. Rook and Campling (1965) reported high concentration of Na⁺ and Cl⁻ in colostrum, decreased and remained steady concentration in early and mid lactation and again increased noticeably towards the end of lactation. By monitoring lactose content of milk during whole lactation Daniel and Pavithran (1985) reported low level of lactose in early stages reached normal level by 7th day of lactation and started declining from day 300 of lactation.

Likewise the false negative results may be attributed to the secondary pathogens
causing only mild inflammatory reactions (Schalm et al., 1971) almost no or very little biochemical changes in milk composition (Bramley, 1975). Further the false negative samples with these tests may be seen in early stages of infection when sufficient changes had not taken place in the milk which were responsible for declaring the particular milk sample as subclinically positive. BTB test may show FN results due to lactose fermenting properties of some of the organisms which may be responsible for neutralizing the increased pH. Besides this acidic pH of milk in early stages of lactation also account for false negative results to some extent.

REFERENCES

ICAR - Sub Committee (1979) Manual in Dairy Chemistry. ICAR, New Delhi, 58 p