Screening of bulk tank milk in fifty seven dairy herds in Tehran suburb

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Abstract
Subclinical mastitis is a serious dairy herd problem. Many infected cows contribute to the low production and high somatic cell count (SCC), which reduce milk quality premiums. Bulk-tank milk analysis is a useful tool for improving milk quality in a dairy operation. A cross-sectional survey of 57 dairy farms in the suburb of Tehran was carried out with sampling in 3 consecutive intervals. Milk samples were cultured and divided in four groups based on total bacterial count (TBC) and SCC. Twenty-six samples had TBC<50000 and SCC<25000 (group 1), 18 samples had TBC≥50000 and SCC<25000 (group 2), 7 samples had TBC<50000 and SCC≥25000 (group 3) and 6 samples had TBC≥50000 and SCC≥25000 (group 4). The microorganisms isolated from the 4 groups in order 1-4 were: Streptococcus agalactiae (4, 0, 0 and 2), Staphylococcus aureus (7, 6, 3 and 1 in 4), high level of Streptococcus uberis (4, 2, 3 and 3 in 4), Streptococcus dysgalactiae (3, 5, 4 and 1), high level Escherichia coli (23, 16, 5 and 4), high level Klebsiella species (2, 1, 0 and 0), high level Enterobacter (3, 2, 4 and 0), high level Citrobacter (10, 13, 5 and 2), high level Campylobacter-negative Staphylococcus (21, 12, 5 and 6), Pseudomonas aerogenes (13, 10, 2 and 5), Proteus (6, 1, 2 and 0), Serratia marcescens (2, 2, 0 and 2), Nocardia asteroides (3, 0, 1 and 1), Listeria monocytogenes (2, 1, 1 and 0) and Edwardsiella tarda (0, 1, 1 and 0). In this study except Proteus and Bacillus, other above mentioned pathogens were isolated in the culture, presence of most these pathogens in BTM indicate that there are problems in our mastitis control programs in our dairy farms. The present screening study may help to design mastitis prevention protocols in the future. Islamic Azad Univ., Garmshir Branch, 4.2.96-98, 2008.

Keywords: bulk tank milk, SCC, TBC
Introduction

Bulk Tank Analysis (BTA) is widely useful screening and monitoring tool which may be used in its entirety or using individual screening tests to monitor specific problem areas. BTA can help pinpoint problem areas in high Bactoscan (TBC) herds, while providing useful information in somatic cell count and clinical mastitis problem herds(3). The methodology of the testing is described in Blowey et al. 1997.

Bulk tank SCC (BTSCC) is a function of the prevalence of IMI within a dairy herd and is a key indicator of milk quality(15) many processors pay quality premiums for low-BTSCC milk, because there is a negative relationship between SCC and casein composition and shelf life of processed fluid milk (3,11). Ott and Novak (12) demonstrated that herds with a BTSCC <200,000 cells/mL attained significantly more profit per cow than herds with a BTSCC ≥400,000 cells/mL.

BTSCC depends on SM and NIR among cows being milked into the bulk tank (7; 15). Observing the BTSCC is a readily available and inexpensive way of monitoring the mastitis trend in the Herd (16).

Standard Plate Count (SPC) is the official regulatory test used for estimating bacterial populations of raw milk and milk products. The SPC is a critical control point for milk quality and many milk purchasers have standards that are more rigorous than the official regulations (13). All the bacteria in milk have originated from the udder, environment or a dirty plant.

The Coliform Count gives an indication of environmental contamination which is commonly due to poor teat preparation or poor hygiene. Coliforms act as a marker for all the environmental organisms such as fecal Strep, Yeasts and Fungi (3).

Pseudomonas Count gives another indication of non-enteric environmental contamination although the source of these organisms is very different. Pseudomonas bacteria are psychrotrophs and so multiply in cold conditions.

Strep uberis, Staph aureus and the Total Staph Counts give a measure of these individual bacteria.

The Total Staph Count measures all Staphs including Staph aureus. These counts are useful in herds with contagious mastitis problems.

Strep uberis is predominantly an environmental bacterium associated with the use of straw bedding and is a common cause of clinical and subclinical mastitis in the UK.

The somatic cell count of milk gives an indication of the level of subclinical mastitis.

The objective of this study describes the process of using of BTM analysis to make decisions on improving milk quality and udder health of the herd.

Material and Methods

Bulk milk samples were taken from fifty seven dairy herds that their milk production and number of milking cows average were 28 kg and 356 cows, respectively. In Tehran suburb, based on their tonnage of milk, for Confirmations the samples were taken in three consequence intervals inside the sterile tubules in hygiene manner and transferred to the laboratory of faculty of veterinary medicine, Islamic Azad University, Karaj branch with ice.

In the laboratory microbiological analysis and somatic cell count performed on the BTM samples, samples on the same day were cultured on Mac conkey agar and blood agar, in case of isolation and detecting of staph. Aureus the samples were frozen and then defrost before microbiological analysis. Therefore for isolating and diagnosis of pathogens in the lab, milk samples were divided in two parts: A, and B. The part A was cultured on blood agar to isolate gram positive and gram negative pathogens and mac conkey agar in order to culture, Part B was specialized for detecting staph aureus as explained above.

It should be mentioned that for counting of somatic cells, BTM samples were smeared on slide during 2 hours after sampling and then counted with manual or automatic method. The method of smearing is so that 0.01 cc or 10, of milk samples were prepared and smeared 1cm x 1cm on slide and after staining with blodomethyl 1%, we count cells with magnitude of 40 or 100; with calculating of average cell count base on considering that the number 1 is equal to 500,000 cells.

The next step of this study was total bacterial
samples had TBC > 50000 and SCC ≤ 250000 (group 4) that has shown in Table 1.

As we know, presence or absence of pathogens in BTM, either number of them more than acceptable thresholds can be able to discuss.

In this study except *Prototheca* and *Bacillus*, other above mentioned pathogens were isolated in the culture. As it is observed, presence of most these pathogens in BTM indicate mastitis or unsanitary problems in the herds. For the pathogens with acceptable threshold we emphasizes on standard plate that implemented in culture media.

### Discussion

All four groups can be investigated as below in order to reach the tools for suspecting the kind of herd problems and use some proper monitoring methods to win against them group 1 (TBC < 50000, SCC > 250000) and group 2 (TBC ≥ 50000, SCC ≤ 250000) both of them have SCC more than 250000 therefore these herds probably suffer from contamination in milking process with contagious mastitis. The isolation rates of *S. aureus* and *S. agalactiae* were significantly associated with BTSCC in BTM (Table 1). Fenlon et al. (4) showed a significant correlation between number of *S. agalactiae*, *S. dysgalactiae*, and *S. albonis* in BTM. *Staphylococcus aureus* was less significantly correlated to BTSCC. Greer and Pearson (6) observed that herds with a higher BTSCC had a higher frequency of isolation of *S. agalactiae*.

It was observed that the mean CNS count was significantly associated with mean BTSCC. Coagulase negative staphylococci are frequently isolated from milk samples and are a significant cause of mild inflammation and elevated cell counts. The CNS generally produce a mild elevation of milk SCC, but if the cows have chronic mastitis, the SCC can elevate to millions (12), this study found that changes in BTSCC do reflect on the CNS count and frequency of isolation of *S. aureus* and *S. agalactiae* from BTM.

In group 2 (TBC < 50000, SCC > 250000) and group 4 (TBC ≥ 50000, SCC ≤ 250000) TBC is more than normal level because of high coliform count (the average of coliform count in three consequence

### Table 1: Proportion of herds based on average TBC and SCC in three consequence intervals in four groups and average percentage of isolated bacteria from three samples more than acceptable thresholds in each group.

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Group 1 Mean</th>
<th>Group 2 Mean</th>
<th>Group 3 Mean</th>
<th>Group 4 Mean</th>
<th>Mean percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leuconostoc</td>
<td>23</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Lactococcus</td>
<td>13</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Klebsiella</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>13</td>
<td>11</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Proteus</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td><em>Corynebacterium</em></td>
<td>20</td>
<td>12</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
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<tr>
<td>Staphylococcus</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Staphylococcal enterotoxigenic</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

count and coliform count. For coliform count the media used was brilliant bile left Cochr to the basis of NPN methods. In counting of total bacteria the diluting of milk for second or third time occasionally is necessary.

### Results

The results of study showed that the more percent of herds are located in range of TBC < 50000 and a few herds are located in range of TBC > 100000 (Figure 1) it is also shown that more percentage of herds involved with the average of SCC between 250000 and 50000 and less related to SCC > 100000 (Figure 2).

Herd milk samples were cultured and divided in four groups based on average total bacterial count (TBC) and average of SCC. Twenty six herd samples had TBC < 50000 and SCC < 250000 (group 1), eighteen herd samples had TBC > 50000 and SCC > 250000 (group 1), seventeen herd samples had TBC < 50000 and SCC ≤ 250000 (group 3) and six herd
sample in six herds was 3, means that more than eleven coliform colony were isolated according to NPN methods.

The S. coagulase negative, environmental streptococci, coliform count, and noncoliform count are collectively termed environmental mastitis pathogens. These organisms gain access to bulk tank milk, not only from intramammary infections, but also from nonspecific contamination from cow skin surface, bedding, manure, and water. The presence of these organisms in BTM may relate to the general level of environmental and milking hygiene in the herd (5). An increase in their numbers in BTM is suggestive of problems related to stall management, udder hygiene, and milking practices (9). Coagulase-negative staphylococci are opportunistic pathogens and form a part of the resident bacterial flora on teat skin. When provided with a favorable opportunity to colonize the teat end or teat canal, they grow to considerable numbers and enter the gland to produce mastitis (14).

Presence of coliform bacteria in BTM milk is suggestive of fecal contamination. Coliforms include *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp. These environmental organisms are frequently isolated from BTM. *Escherichia coli* in particular have been shown to elevate bacterial numbers in BTM (8).

Presence of *nokardia*, *pseudomonas* and *serrata mareeccense* can indicates problems in water that used in herds or contaminant udder ointment that should be consider more.

At the end it should be emphasized that, the present screening study may help to design mastitis prevention protocols in the future.

**References**

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