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

Ebrahimi, A.[1]*, Farhoodi moghaddam, M.[2], Tajik, P.[3], Akbari, G.[3]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar-Iran.

[2]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj-Iran.

[3]Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran.

Corresponding Author: Email: aebrahimy@ut.ac.ir

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>250000 (group 1), 18 samples had TBC≥50000 and SCC>250000 (group 2), 7 samples had TBC<50000 and SCC≤ 250000 (group 3) and 6 samples had TBC≥ 50000 and SCC≤250000 (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 *Coagulase-negative Staphylococcus* (21, 12, 5 and 6), *Pseudomonas aerogenese* (13, 10, 2 and 5), *Proteus* (6, 1, 2 and 0), *Serratia marcescens* (2, 2, 0 and 2), *Nocardia astroides* (3, 0, 1 and 1), *Listeria monocytogenes* (2, 1, 1 and 0) and *Edwardsiella tarda* (0, 1, 1 and 0). In this study except *Prototheca* 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., Garmsar Branch. 4,2:94-98,2008.***

Keywords: bulk tank milk,SCC,TBC

آنالیز شیر مخزن کل ۵۷ گاو داری اطراف تهران

امین ابراهیمی[1*]، مهران فرهودی مقدم[2]، پرویز تاجیک[3]، قاسم اکبری[3]

۱- گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه آزاد اسلامی واحد گرمسار، گرمسار - ایران. ۲- گروه علوم درمانگاهی دانشکده دامپزشکی، دانشگاه آزاد اسلامی واحد کرج، کرج - ایران. ۳- گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه تهران، تهران - ایران.

چکیده

وجود ورم پستان تحت بالینی یکی از مشکلات گله های شیری است، که باعث کاهش تولید وهمچنین کاهش کیفیت شیر به جهت افزایش تعداد سلولهای سوماتیک می شود. آنالیز مخزن شیر بعنوان ابزار مناسب در پایش وضعیت سلامت پستانها در سطح گله مورد استفاده قرار می گیرد. اطلاعات مربوط به آنالیز تانک شیر به حل مشکل ورم پستان در سطح گله و در نهایت بهبود کیفیت شیر کمک می کند. یک مطالعه متقاطع بر روی ۳ نمونه شیر متوالی مربوط به تانک شیر ۵۷ گله شیری اطراف تهران صورت گرفت. نمونه های شیر کشت داده شدند و بر اساس شمارش کلی باکتری ها و سلول های سوماتیک به ۴ گروه تقسیم شدند، ۲۶ نمونه دارای TBC<50000 و SCC>250000 بودند (گروه۱)، ۱۸ نمونه دارای TBC≥50000 و SCC>250000 بودند (گروه۲)، ۷ نمونه دارای TBC<50000 و SCC≤250000 بودند (گروه۳) و ۶ نمونه دارای TBC≥50000 و SCC≤250000 بودند (گروه۴). بترتیب ۱ تا ۴ میکروارگانیسم های جداشده از ۴ گروه شامل: استرپتوکوک آگالاکتیه (۴، ۰، ۰ و ۲)، استافیلوکوکوس ارئوس (۷، ۶، ۳، ۱)، وسطح بالایی از استرپتوکوکوس یوبریس (۴، ۰، ۰ و ۳)، استرپتوکوکوس دیسکالاکتیه (۳، ۵، ۴ و ۱)، میزان بالایی از *E. coli* (۲۳، ۱۶، ۵ و ۴)، گونه های کلبسیلا (۲، ۱، ۰ و ۰)، میزان بالایی از انتروباکتر (۳، ۲، ۴ و ۰)، تعداد زیاد سیتروباکتر (۱۰، ۱۳، ۵ و ۲)، میزان زیاد استافهای کواگولاز منفی (۲۱، ۱۲، ۵ و ۶)، سودوموناس آئروژینوزا (۱۳، ۱۰، ۲ و ۵)، پروتئوس (۶، ۱، ۲ و ۰)، سراتیا مارسسنس (۲، ۲، ۰ و ۲)، نوکاردیا آستروئیدس (۳، ۰، ۱ و ۱)، لیستریا مونوسایتوژن (۲، ۱، ۱ و ۰) و اردواردزیلا تاردا (۰، ۱، ۱ و ۰). در این مطالعه بغیر از پروتکا و باسیلوس، سایر پاتوژن هایی که در بالا ذکر شدند در محیطهای کشت جداشدند در نتیجه این مطالعه نشان داد که مونیتورینگ ورم پستان و همچنین اجرای برنامه های کنترلی در گله های ما به درستی انجام نمی شود. این مطالعه غربالی ممکن است در آینده به برنامه های پیشگیری ورم پستان در سطح گله کمک کند. مجله دانشکده دامپزشکی دانشگاه آزاد اسلامی واحد گرمسار، ۱۳۸۷، دوره ۴، شماره ۲، ۹۸-۹۴.

واژه های کلیدی: شیر مخزن کل، شمارش کلی سلول های سوماتیک، شمارش کلی میکروبی.

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 (10).

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 faecal Streps, 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 psychotrophs 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 350 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.areus 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 10λ of milk samples were prepared and smeared 1cm×1cm on slide and after stating with blodomethylen 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

Table1. 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.

Pathogens	Group1 Mean percent	Group2 Mean percent	Group3 Mean percent	Group4 Mean percent
E.coli	23	16	5	4
S.uberis	4	2	3	3
S.dysgalactiae	3	5	3	1
Citrobacter	10	13	5	2
Enterobacter aerogenes	3	2	4	—
Klebsiella	2	1	—	—
Pseudomonas aeroginase	13	10	2	5
Proteus	6	1	2	—
S coagulase negative	21	12	5	6
S.agalactiae	4	2	—	2
S.aureus	7	6	3	1
Listeria monocytogene	2	1	1	—
Nocardia asteroids	3	—	1	1
Serratia marcesence	2	2	—	2
Edwadsella tarda	—	1	1	—

count and coliform count. For coliform count the media used was brilliant Bile Colin Broth on 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>1000000 (figure 1) it is also was shown that more percentage of herds involved with the average of SCC between 250000 and 500000 and less related to SCC>1000000 (figure2)

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 (group1), eighteen herd samples had TBC≥50000 and SCC>250000 (group2),seven herd samples had TBC <50000 and SCC≤250000 (group3) and six herd samples had TBC≥50000 and SCC≤250000 (group4) that has shown in table1.

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 group1 (TBC<50000, SCC>250000) and group2 (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. uberis* 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 group2 (TBC≥50000, SCC>250000) and group4 (TBC≥50000,SCC≤250000) TBC is more than normal level ,because of high coliform count (the average of coliform count in three consequence

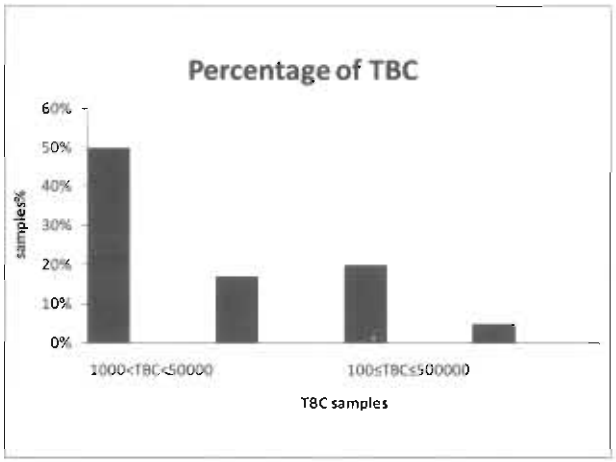

Fig 1. Proportion of herds involved with different range of average TBC in three consecutive intervals.

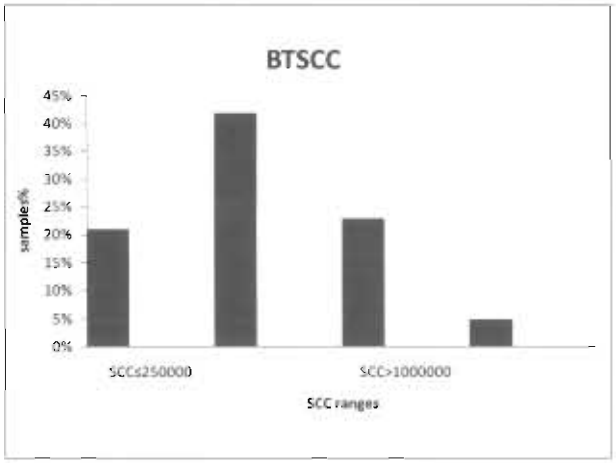

Fig 2. Proportion of herds involved with different range of average SCC in three consecutive intervals.

sample in six herds was +3, means that more than eleven coliform colony were isolated according to NPN methods)

The S. coagolase 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 *serratia marececcense* 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

1. Andrews, A. T., Cheeseman,G. C. (1980) Influence of elevated somatic cell count on casein distribution and cheesemaking. J. Dairy Res., **47**:393-400.
2. Eberhart, R. J., Hutchinson,L. J., Spencer, S. B. (1982) Relationship of bulk tank somatic cell counts to prevalence of intramammary infection and indices of herd production. J. Food Prot., **45**:1125-1128.
3. Edmondson, P. (2005) Practical use of Bulk Tank Analysis for Mastitis AND Milk Quality Problems, NMC Annual Meeting Proceedings
4. Fenlon, D. R., Logue, D. N., Gunn, J., Wilson, J. (1995) Management practices to identify their relationship to high somatic cell counts in bulk tank milk. Br. Vet. J., **151**:17-25.
5. Godkin, M. A., Leslie, K. E. (1993) Culture of bulk tank as a screening test: A brief review. Can. Vet. J., **34**:601-605.
6. Greer, D. O., Pearson, J. K. L. (1973). Streptococcus agalactiae in dairy herds. Its incidence and relationship to cell count and inhibitory substance levels in bulk milk. Br. Vet. J., **129**:544-553.
7. Harmon, R. J. (1994) Physiology of mastitis and factors affecting somatic cell counts. J. Dairy Sci., 77:2103-2112.
8. Hayes, M. C., Ralyea,R. D., Murphy,S. C., Carey,N.

R., Scarlett,J. M. Boor, K. J. (2001) Identification and characterization of elevated microbial counts in bulk tank milk. J. Dairy Sci., **84**:292- 298.

9. Jayarao, B. M., Wolfgang, D. R. (2003) Bulk-tank milk analysis. A useful tool for improving milk quality and herd udder health in Update on Bovine Mastitis, Vet. Clin. N. Am. Food Anim. Pract., **19**: 75-92
10. Lukas J. M., Hawkins, D. M., Kinsel, M. L., Reneau J. K. (2005) Bulk Tank Somatic Cell Counts Analyzed by Statistical Process Control Tools to Identify and Monitor Subclinical Mastitis Incidence. J. Dairy Sci., **88**:3944-3952.
11. Ma, Y., Ryan, C., Barbano, D. M., Galton, D. M., Rudan, M. A., Boor, K. J. (1999) Effects of somatic cell count on quality and shelflife of pasteurized fluid milk. J. Dairy Sci., **83**:264-274.
12. Ott, S. L., Novak, P. R. (2001) Association of herd productivity and bulk-tank somatic cell counts in US dairy herds. J. Am. Vet. Med. Assoc., **218**:1325-1330
13. Ruegg, R.L., Reinemann, D.J. (2002) Milk Quality and Mastitis Tests .University of Wisconsin Madison.
14. Sears, P. M., McCarthy, K. K. (2003) Management and treatment of staphylococcal mastitis. in Update on Bovine Mastitis, Vet. Clin. N. Am. Food Anim. Pract., **19**:171-185.
15. Wenz, J. R., Jensen, S. M., Lombard, J. E., Wagner, B. A., Dinsmore, R. P. (2007) Herd Management Practices and Their Association with Bulk Tank Somatic Cell Count on United States Dairy Operations. J. Dairy Sci., **90**:3652-3659.