# Bulk tank milk somatic cell count and sources of raw milk contamination with mastitis pathogens

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**ABSTRACT**: The objective of this study was to probe the relationship between prevalence of selected principal mastitis pathogens and somatic cell counts in bulk tank milk samples. The sources of milk contamination were evaluated. The samples were collected from 298 dairy herds (with approximately 32 000 dairy cows). Only 48.3% of the bulk tank milk samples were free of contamination of pathogens of interest. Approximately 38.9% of the milk samples were contaminated with only one, 12.4% with two and 0.3% with three pathogens. The arithmetic mean of logarithmically transformed data of bulk tank milk somatic cell count rise in order: pathogen free, *Pseudomonas aeruginosa*, *Streptococcus uberis*, *Escherichia coli* and *Staphylococcus aureus* (5.381; 5.413; 5.495; 5.518; 5.563, respectively). The arithmetic mean differences between bulk tank milk somatic cell counts in pathogen-free and single-pathogen contaminated samples have revealed a significance for the *Escherichia coli* and *Staphylococcus aureus* groups ($P < 0.01$). Using binary logistic regression, a statistically highly significant relationship ($P < 0.001$) has been found between the number of contaminations of bulk tank milk samples with mastitis pathogens and bulk tank milk somatic cell counts. The relationship allows the determination of the probability of finding relevant mastitis pathogens in bulk tank milk samples with different levels of bulk tank milk SCC. A 63% probability can be defined at a cell count level of 400 000/ml and 20% at a cell count level of 100 000/ml. Analysis may reveal the potential sources of the bulk tank milk sample contamination, i.e. infected mammary glands or the environment. The presence of high levels of contamination along with a low bulk tank SCC may suggest an environmental source of contamination. The study clarified that a potential source of bulk tank milk contamination by relevant pathogens (the environment or the mammary gland) may be elucidated and the probability of the contamination of bulk tank milk samples with mastitis pathogens predicted by the analysis of relationship between the bulk tank milk somatic cell counts and the number of mastitis pathogen contaminations.

**Keywords**: bulk tank milk samples; somatic cell count (SCC); *Streptococcus uberis*; *Pseudomonas aeruginosa*; *Escherichia coli*; *Staphylococcus aureus*

Good udder health is essential for quality milk production. Somatic cell count (SCC) is the most widely accepted criteria for measuring udder health and milk quality in all major milk-producing countries throughout the world.

Bulk tank milk SCC (BTMSCC) is a function of the percentage of quarters infected by major pathogens in a dairy herd. Eberhart et al. (1982) reported a linear relationship between BTMSCC and the percentage of quarters infected with major pathogens. Using this relationship, the percentages of quarters infected at BTSCC levels of 200 000; 400 000; 750 000; and 1 000 000 cells/ml were reported to be 6.2%; 12.8%; 24.3%; and 32.6%, respectively.

BTMSCC is a better tool for monitoring mastitis caused by contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, than mastitis caused by environmental pathogens such as *Streptococcus uberis* and *Escherichia coli* (Smith, 1996). A higher correlation was determined between the percentage of intramammary infection (IMI) caused by *S. agalactiae* and the BTMSCC than of *S. aureus* IMI, respectively

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(Pastrnkova and Rysanek, 1984; Fenlon et al., 1995). The correlation ($r$ = 0.87) between the herd bulk milk somatic cell count (SCC) and its estimation from the quarter milk somatic cell count allowed to evaluate the contribution of the different quarters, according to their infection status, to the herd bulk milk SCC. Quarters infected by a major pathogen (8.5% of samples) gave rise to 46.6% of the total number of cells, while quarters infected by coagulase-negative staphylococci (17.8% of samples) gave rise to 18.1% (Rainard et al., 1990).

It is known that the prevalence of contagious pathogens causing mastitis is decreasing and environmental causative agents are becoming dominant pathogens for the mammary gland (Hillerton and Berry, 2003). Reports from United States of America and from Great Britain indicate creating role of environmental mastitis pathogens. Coliforms and streptococci other than *S. agalactiae* accounted for 94% of the major pathogen infections (Oliver, 1988). Contagious pathogens were isolated only in 3.4% of clinical mastitis cases in well-managed herds (Hogan et al., 1989). It has been estimated that contagious mastitis pathogens represent less than one third of all mastitis cases compared to > 75% of all cases 20 years ago (Hillerton et al., 1995). Environmental streptococci, coliforms and coagulase-negative *Staphylococcus* spp. were the most commonly isolated pathogens (Hoe and Ruegg, 2005).

In United States of America many practitioners have successfully incorporated bulk tank milk (BTM) pathogens culturing into herd monitoring procedures despite there is no standard for this. A procedure successfully used was outlined by National Mastitis Council (2005).

The objective of this study was to probe whether an analysis of the relationship among the BTMSCC and the prevalence of selected pathogens of the mammary gland could indicate the source of BTM contamination. The purpose of study was to contribute to interpretation of the BTM culturing results.

## MATERIAL AND METHODS

### Experimental design

Bulk tank milk samples were collected from 298 dairy herds (with approximately 32 000 dairy cows) on regular test days scheduled for the quality determination of milk hygiene in the period from April to July 2005. The samples were taken in the area of North Moravia and East Bohemia.

The prevalence of four principal mammary gland pathogens and SCC was determined. The frequency and combinations of the microbiological findings were analysed. Differences were determined between the BTMSCC in groups of pathogen-free and single-pathogen contaminated samples. The binary logistic regression for the contamination of BTMS with relevant pathogens and BTMSCC was calculated and the probability of finding pathogens was determined. The relationship between BTMSCC and results of bacterial culturing of mastitis pathogens was used for judgement of the sources of BTM bacterial contamination.

### Sampling procedure

The BTMS intended for checking the hygienic quality of raw milk were collected in accordance with European Standard EN ISO 707:1997, mostly using auto-samplers (AutoSampler, Foss Electric, Hillerod, Denmark), preserved with Heeschen's reagent (Heeschen et al., 1969), transported to the laboratory in polystyrene boxes containing freezer packs and examined within 24 h of collection. The technical design of polystyrene boxes rule out the samples been frozen. The samples were collected by specially educated technicians from the bulk tank milk of raw milk suppliers. Quality parameters (especially the total bacteria count and SCC) were determined by the laboratory of Mlekolab Pardubice, s.r.o., Czech Republic. Thereafter, the samples were frozen and transported to our laboratory, where they were thawed (at laboratory temperature), carefully mixed by swinging and cultured.

### Microbiological examination

*Streptococcus uberis* detection was carried out by the inoculation (about 0.050 ml) of milk samples using a glass rod on Edwards medium (OXOID, Basingstoke, England). After 24 h of incubation at 37°C, aesculin-positive colonies with typical morphology were isolated and transferred to blood agar (BA). The catalase test (Chemical Company Sokolov, Sokolov, Czech Republic) and Christie, Atkins and Munch-Petersen (CAMP) test were performed, and the growth capability on agar contain-

ing 6.5% NaCl supplemented with bile was assessed. Furthermore, a test for sodium hippurate hydrolysis and PYR test (detection of pyrrolidonyl arylamidase) (PLIVA-Lachema, Brno, Czech Republic) were conducted. Suspect strains of *S. uberis* were tested by STREPTOtest (PLIVA-Lachema, Brno, Czech Republic).

*P. aeruginosa* detection was performed by the inoculation of 0.2 ml milk sample smears on Cetrimide-agar (MERCK, Darmstadt, Germany). After 48 h of incubation at 35°C, the agar was evaluated using a UV lamp (366 nm). Typical colonies were subcultured on BA and after 24 h of incubation at 37°C, an OXI test (PLIVA-Lachema, Brno, Czech Republic) was performed; OXI-positive strains were inoculated on Simmons citrate agar, on Motility Test Medium (HIMEDIA, Mumbai, India) and on MacConkey agar (MERCK, Darmstadt, Germany).

*E. coli* detection was performed by the inoculation of 0.1 ml milk sample smears on MacConkey agar (MERCK, Darmstadt, Germany). After 24 h of incubation at 37°C, five lactase-positive colonies were marked and selected. These colonies were isolated by subculture on blood agar (BA). If fewer than five lactase-positive colonies grew, they were all collected for isolation. After 24 h of incubation, the cultures were tested by the OXI test (PLIVA-Lachema, Brno, Czech Republic). OXI-negative strains and controls were inoculated on Simmons citrate agar (MERCK, Darmstadt, Germany) and Motility Test Medium (HIMEDIA, Mumbai, India) and incubated for 24 h at 37°C. After their assessment, a COLI test (PLIVA-Lachema, Brno, Czech Republic) was carried out.

*S. aureus* detection was performed by the inoculation of 0.1 ml milk sample smears on Mannitol Salt Agar (OXOID, Basingstoke, England). After 36 h of incubation at 35°C, typical colonies were subcultured on BA and incubated 24 h at 37°C. Catalase test (Chemical Company Sokolov, Sokolov, Czech Republic) and tube coagulase test (ITEST, Hradec Kralove, Czech Republic) were conducted. Coagulase-positive strains were examined by a VPtest (Voges-Proskauer test) (PLIVA-Lachema, Brno, Czech Republic) and a Latex agglutination test (ITEST, Hradec Kralove, Czech Republic).

### Somatic cell counts

The somatic cell counts were estimated using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark) with a procedure in accordance with EN ISO Standard 13366-3:1997.

The results of the Mlekolab laboratory were used in our following analysis.

### Statistical analysis

The raw data were pre-processed with our own software created by the compiler Borland Pascal 7.0 (Borland International Inc., Scotts Valley, CA, USA). Using this software, we first tested the normality of raw data (BTMSCC) distribution. The Anderson-Darling test (Anderson and Darling, 1952) showed the raw data had a distribution which is skewed to the right. In order to be able to work with normal distribution data, we carried out a logarithmic transformation (with logarithm base 10).

The descriptive statistics of the transformed data were assessed by means of Data Analysis ToolPack (DATP), as a part of Microsoft Excel 2003 (Microsoft Corp., Redmond, Washington D.C., USA) software. The significance of the arithmetic mean differences between the logBTMSCC in groups of pathogen-free and single-pathogen contaminated samples was determined by a two-tailed unpaired *t*-test (DATP). The relevant test (with equal/unequal variances) was chosen with regard to the test of the homogeneity of variances at a level of 0.05 (*F*-test two-sample for variances, DATP).

The binary logistic regression was performed with our own software created by the compiler Borland Pascal 7.0 in accordance with the recommendations of Hosmer and Lemeshow (2000).

## RESULTS

As demonstrated in Table 1, only 48.3% of the bulk tank milk samples were free of contamination with the mammary gland pathogens under study. Approximately 38.9% of the milk samples were contaminated with only one of the selected pathogens, 12.4% with two and 0.3% with three pathogens. The arithmetic mean of the logBTMSCC (Table 2) increases in sequence from pathogen-free and single-pathogen to double-pathogen contaminated milk samples.

The arithmetic mean of the logBTMSCC (Table 2) increase in this order: pathogen-free, *P. aeruginosa*, *S. uberis*, *E. coli* and *S. aureus* contaminated bulk tank milk samples, i.e. from free and environmental

Table 1. Findings of mastitis pathogens and somatic cell counts in bulk tank milk samples

| Pathogen findings | | Numer of samples | Per cent | BTMSCC (log) | BTMSCC (× $10^3$/ml) |
|---|---|---|---|---|---|
| Negative | | 144 | 48.32 | 5.3811 | 240.0 |
| Single | EC | 63 | 38.93 | 5.5195 | 330.8 |
| | SA | 32 | | | |
| | SU | 11 | | | |
| | PA | 10 | | | |
| Double | EC + SA | 17 | 12.42 | 5.5654 | 367.6 |
| | EC + PA | 9 | | | |
| | EC + SU | 7 | | | |
| | SA + SU | 2 | | | |
| | SA + PA | 2 | | | |
| Triple | SA + EC + PA | 1 | 0.33 | – | – |
| Total | | 298 | 100.00 | 5.4577 | 286.9 |

Negative = pathogen-free samples; EC = *Escherichia coli*; SA = *Staphylococcus aureus*; SU = *Streptococcus uberis*; PA = *Pseudomonas aeruginosa*

to contagious pathogens, respectively. Analysis of the arithmetic mean differences between the log-BTMSCC in pathogen-free and single-pathogen contaminated samples have revealed (Table 2) a significance only for the *E. coli* and *S. aureus* groups of contaminated samples ($P < 0.01$).

Using binary logistic regression, a statistically high significant relationship ($P < 0.001$) has been found between a number of contaminations of BTMS with mastitis pathogens of interest and the BTMSCC (Figure 1). This relationship enables determining the probability of findings for the relevant mastitis pathogens in BTMS at different levels of the BTMSCC. For example, 63% probability can be definite at a cell count level of 400 000/ml; 20% at a cell count level of 100 000/ml.

Table 2. Significance of differences between means of bulk tank milk somatic cell counts (logBTMSCC) in groups of pathogen-free samples and single pathogen contaminated milk samples

| | Negative | PA | SU | EC | SA | Overall |
|---|---|---|---|---|---|---|
| Number | 144 (55.38%) | 10 (3.85%) | 11 (4.23%) | 63 (24.23%) | 32 (12.31%) | 260 (100.00%) |
| Mean | 5.3811 | 5.4134 | 5.4951 | 5.5183 | 5.5635 | 5.4428 |
| SD | 0.2408 | 0.1379 | 0.2258 | 0.2066 | 0.1721 | 0.2333 |
| CV | 4.4754 | 2.5477 | 4.1085 | 3.7447 | 3.0939 | 4.2866 |
| Minimum | 4.2788 | 5.1239 | 5.1644 | 5.0934 | 5.1790 | 4.2788 |
| Maximum | 5.9809 | 5.5855 | 5.9921 | 6.2460 | 5.9768 | 6.2460 |
| $95^{th}$ percentile | 5.7641 | 5.5691 | 5.8524 | 5.8696 | 5.9184 | 5.8248 |
| $99^{th}$ percentile | 5.8624 | 5.5822 | 5.9642 | 6.0351 | 5.9751 | 5.9785 |
| Significance | – | $P > 0.05$ | $P > 0.05$ | $P < 0.01$ | $P < 0.01$ | – |

Overall = all values of logBTMSCC; Negative = pathogen-free; PA = *Pseudomonas aeruginosa*; SU = *Streptococcus uberis*; EC = *Escherichia coli*; SA = *Staphylococcus aureus*; SD = standard deviation; CV = coefficient of variation
Significance = unpaired *t*-test (differences positive vs. negative)

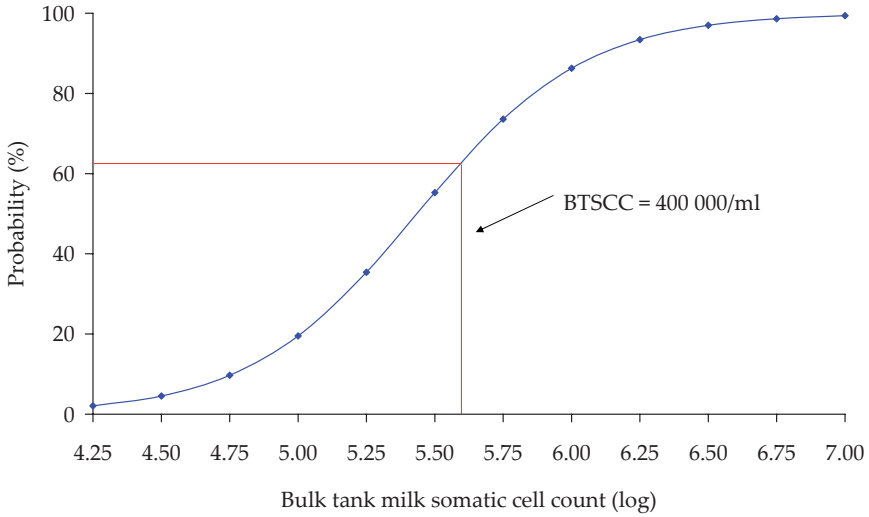


Figure 1. Probability of findings for mastitis pathogens in bulk tank milk samples based on somatic cell count – binary logistic regression

Characteristics of regression: Mean of bulk tank milk somatic cell count in logarithm (log BTMSCC) = 5.4577; SD (standard deviation [log]) = 0.2406; N–/N+ (number of samples without/with finding of mastitis pathogens) = 144/154; Intercept/Slope (coefficients of logit function) = –17.6882/3.2545; SES (standard error of slope) = 0.6588; *P* (two-tailed probability value for slope) = 0.0010; Overall Model Fit Significance = $P < 0.001$)

## DISCUSSION

Various studies investigated risk factors in herds and their associations with BTMSCC (Erskine et al., 1987; Schukken et al., 1991; Wilson et al., 1995, 1997; Barkema et al., 1998a; Barkema et al., 1999; Khaitsa et al., 2000; Peeler et al., 2000; McDougall, 2003; Barnouin et al., 2004; Rodrigues et al., 2005; van Schaik et al., 2005; O'Reilly et al., 2006). Attention was also focused on the correlation between the incidence of subclinical or clinical mastitis and BTMSCC (Barkema et al., 1998b; McDougall, 2003; Lukas et al., 2005). Few authors concentrated on the culture findings of pathogens of the bovine mammary gland in BTMS and BTMSCC simultaneously (Fenlon et al., 1995; Jayarao et al., 2004; Olde Riekerink et al., 2006) as we did in the present study.

The objective of this study was to probe the relationship between the prevalence of selected mastitis pathogens and somatic cell count in bulk tank milk samples. It is surprising that the BTMSCC increases from pathogen-free and single-pathogen to double-pathogen contaminated BTMS in spite of the classification in environmental or contagious pathogens. Therefore we assume that it indicates the great importance of environmental pathogens in current infections of the mammary glands. This discovery found a high number of *E. coli* findings as contamination agents of BTMS and a significant higher BTMSCC in BTMS contaminated with *E. coli* in contrast to the uncontaminated samples. On the other hand, a higher percentage of BTMS contamination with *S. aureus* and a contemporary high BTMSCC is not surprising. It indicates that *S. aureus* remains the principal mastitis pathogen under current conditions in the Czech Republic.

Fenlon et al. (1995) revealed a good correlation between the number of mastitis streptococci (*S. agalactiae*, *S. dysgalactiae* and *S. uberis*) found in bulk tank milk and the BTMSCC. *Staphylococcus aureus* was less significantly correlated to BTMSCC. However we evaluated the samples in a different way than Fenlon et al. (1995); we must say that the results obtained were comparable only for *S. aureus*. *S. uberis* alone was not a specific interest of Fenlon et al. (1995).

The objective of the Olde Riekerink et al. (2006) article was to estimate the herd prevalence of contagious mastitis pathogens in bulk milk from Prince Edward Island dairy farms and determine the association between bulk milk culture results and mean BTMSCC. The cumulative prevalence of *S. aureus*, *S. agalactiae* and *Mycoplasma* spp.

(*M. bovis* and *M. alkalescens*) was 74%, 1.6% and 1.9%, respectively. The BTMSCC of *S. aureus*-positive herds was higher than that of negative herds, which is fully in accordance with the finding of the present study.

The contamination with two major pathogens, *S. uberis* and *P. aeruginosa* was not accompanied by elevated BTMSCC in this study. We believe that this discovery could make it possible to differentiate between sources of BTMS contamination. A high percentage of major pathogen contamination of BTMS accompanied with a low level of BTMSCC shows environmental contamination of BTMS. Both relevant pathogens accompanied with a low level of BTMS in this study belong among environmental mammary gland pathogens. Considering this, it is good to know (Pastrnkova and Rysanek, 1984; Rainard et al., 1990; Smith, 1996; Wilson et al., 1997) that the relationship between the percentage of IMI and BTMSCC depends on the severity of the somatic cell response to infection by the relevant pathogen; it is necessary to take this into account. For example, a low correlation was determined between the BTMSCC and environmental streptococci bacteria count (Jayarao et al., 2004).

The relationship allows the determination of the probability of finding relevant mastitis pathogens in bulk tank milk samples with different levels of bulk tank milk SCC. Our observation is in accordance with the results of Jayarao et al. (2004), that an increase in the frequency of isolation of *S. aureus* and *S. agalactiae* in BTMS was significantly associated with an increased BTMSCC.

The procedure of culturing BTMS to monitor milk quality is regulated by international standards (Commission Regulation (EC) No 1662/2006 amending Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin). In contrast, no standard is available for BTMS culturing for the detection of mastitis pathogen contamination. Various authors were used different media for this purpose (Fenlon et al., 1995; Benda and Vyletelova, 1997a,b; Jayarao and Wang, 1999; Hayes et al., 2001; Sawant et al., 2002; Jayarao et al., 2004; Zadoks et al., 2004; Olde Riekerink et al., 2006). We have used culture media similar to the recommendation of the National Mastitis Council (2005) for the purpose of this study. However, we are aware of certain limitations while using BTMS for diagnosis of mastitis pathogens, for example the contamination of BTM from the environment and equipment.

Nevertheless, this study clarified that a source of BTM contamination by relevant pathogens (the environment or the mammary gland) may be elucidated. We have come to the conclusion, that a high percentage of BTMS contamination with the relevant pathogens and the coincident low level of BTMSCC indicate an environmental source of BTMS contamination.

Howard (2006) did not study the association between the counts of selected bacteria and BTMSCC. Nevertheless, the author reported: Interpretation of results based on BTM is difficult as aesculin-positive streptococci, CNS and coliforms can be isolated from the environment as well as from cows with clinical or subclinical mastitis. We believe that the interpretation suggested in the present study, i.e. that a high percentage of BTMS contamination with the relevant pathogens and the coincident low level of BTMSCC indicate an environmental source of BTMS contamination, contributes to the solution of the outlined problem. We agree with the author that more extensive study is required.

## CONCLUSION

This study has detected that not all contaminations of BTMS with major mastitis pathogens cause a coincident elevated level of BTMSCC. The differences between the mean of log BTMSCC in pathogen free and single-pathogen contaminated samples have revealed significance for the *E. coli* and *S. aureus* groups of contaminated samples. The mean of the logBTMSCC increase in order: pathogen free, *P. aeruginosa*, *S. uberis*, *E. coli* and *S. aureus* contaminated BTMS, respectively. A high significant relationship has been certified between the number of contaminations of BTMS with mastitis pathogens and BTMSCC, making it possible to determine the probability of findings for relevant mastitis pathogens in BTMS at different levels of the BTMSCC. The probability ranged from 20% to 63% at cell count levels of 100 000 and 400 000/ml in BTMS, respectively. The analysis used in this study may reveal the sources of BTMS contaminations, i.e. infected mammary glands or the environment. The high percentage of BTMS contamination with the relevant pathogens and the coincident low level of BTMSCC indicate an environmental source of BTMS contamination (*P. aeruginosa* and *S. uberis* in this study).

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