Investigation of udder health and milk quality parameters of dairy farms in Northern Cyprus.

Part I: SCC and bacteriologic examination

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Summary: The aim of this study was to determine herd udder health and milk quality status of all 138 dairy farms in Northern Cyprus. For this purpose, somatic cell counts were measured, and bacteriological isolations were performed monthly for one year in bulk tank milk belonging to 138 dairy farms. The median value of bulk tank milk somatic cell counts was calculated as 521.583 cells/ml (>400,000 cells/ml). After bacteriological isolation and identification, Coagulase Negative Staphylococci from 22.73%; Bacillus spp. from 18.68%; S. aureus from 16.55%; S. dysgalactiae from 11.53%; S. uberis from 7.62%; E. coli from 7.44%; Micrococcus spp. from 1.81%; Pseudomonas spp. from 1.49%; Enterobacteriaceae spp. from 0.90%; Proteus spp. from 0.85%; Aeromonas spp. from 0.58%; Yeast from 0.53%; Pasteurella spp. from 0.47%. In conclusion, it was therefore determined that there are important health problems in the dairy farms of Northern Cyprus in terms of udder health.

Keywords: Bacteriological examination, dairy farm, Northern Cyprus, somatic cell count, udder health.

Introduction

Globally, there have been a general increase in the importance of udder health control programs in cows, in recent years (33). The consumption of milk and dairy products has increased greatly over recent years, and the notion of quality products has come to the fore. Excessive milk consumption, disclosure of animal diseases and epidemic illnesses of animals into public domain have recently increased consumer concerns over food quality (29).

Mastitis is one of the most important problems in dairy farms and it causes huge economic losses. Mastitis control program is a very important issue in terms of reducing economic losses and prevention of mastitis in dairy herds (1). Mastitis control programs can differ from country to country due to some factors such as differences in the prevalence of pathogens, management and environmental circumstances (20). For this reason, herd-level somatic cell counts (SCCs), mastitis causing pathogens and their prevalence should be well known for detailed and comprehensive control program. There are some studies conducted in the USA (17), UK (6), Mexico (18), Canada (7, 27), Norway (25), Finland (23), Estonia (9, 36), Poland (34), Germany (39), Netherlands (32), and

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Denmark (12) to estimate herd and national-level mastitis pathogens, mastitis incidence and bulk tank milk somatic cell count. However, there are no studies for Northern Cyprus.

Analyses of bulk tank milk have recently been used specifically in veterinary medicine to observe herd udder health and milk quality (14). Bulk tank milk analyses have become the most important method in revealing milk quality and udder health in dairy herds (14, 18, 27). During the past three decades, raw milk bacteriology and farm management practices on mastitis, milking and milk hygiene have increased to an important level. Studies in the past decade showed that bulk tank milk analysis is a practical method to monitor and solve many problems (14).

The objective of this study was to investigate the status and changes in herd-level mastitis pathogens, mastitis incidence and bulk tank milk somatic cell count (BTMSCC) within a year in dairy farms in North Cyprus.

Materials and Methods

Study design: This study was conducted between October 2009 and September 2010 and used bulk tank milk samples from 138 dairy farms (all farms in Northern Cyprus in that period, containing 15.552 Holstein cows in total) in Northern Cyprus. Samples were taken from the farms on a monthly basis for one year and 1643 bulk tank milk samples were examined throughout the whole period of the study (at the beginning of the study, number of farms was 134 and reached 138 until the end of the study. Therefore, 1643 bulk tank milk samples were collected). SCCs of all samples were determined and bacteriological isolation was performed.

Sampling: Milk samples were collected using a sterile dip cup from the tank which was mixed well after milking in the morning and sent to the laboratory at +4 °C. Disposable gloves were used during collecting the samples, and the samples were taken into 20 ml sterile tubes. One of the 3 sterile 20 ml milk bottles taken from the farms was frozen at -20 °C, and brought to the laboratory.

Determining the somatic cell counts: The SCC measurements were performed using a Fossomatic TM FC 5000 (Foss, Denmark) device at Cyprus Turkish Milk Industry, Quality Control Department Laboratory.

Bacteriological procedures: Bacteriological examinations of the milk samples were performed in accordance with the protocols set out by the National Mastitis Council, USA (NMC) (10). According to the protocol, samples were vortexed and homogenized at room temperature and then, using a sterile loop, transplanted to Blood Agar containing 5-7% sheep blood, Edward’s Agar, Mac Conkey Agar, and Chapman Agar (0.05 ml). Blood, Mac Conkey and Edward’s Agars were left to incubate for 24-48 hours at 37 °C; and Chapman agars were left to incubate for 48 hours for isolation of S. aureus and Coagulase Negative Staphylococci (CNS) species. Colonies obtained from blood agar during bacteriological isolation were stained with Gram’s staining. Catalase and oxidase tests were applied after the Gram staining, and general bacteriological and yeast identifications were performed. Colonies reproduced in Mac Conkey Agar were evaluated according to their lactose positive and negative properties. Catalase and oxidase tests were applied to the bacteria isolated on blood agar from bacteria colonies isolated as lactose positive, and E. coli was identified. Pseudomonas spp. which led to blue-green pigmentation in Mac Conkey Agar and gave a fruit aroma due to amino-acetophenone in its structure were also identified according to their catalase and oxidase characteristics. Streptococcus spp., isolated from Edward’s Agar after 48 hours were identified according to sodium hippurate tests conducted using ninhydrin reagent and according to Esculin resolutions. Colonies reproduced in Chapman Agar were evaluated according to pigment presence as follows; those possessing yellow pigments were evaluated as S. aureus; and those which had white pigments were evaluated as CNS.

Collecting the meteorological data: In order for the obtained data to be evaluated according to climatic conditions, meteorological data (temperature, relative humidity, and rainfall data) were provided by Northern Cyprus, Ministry of Public Works and Transportation, Meteorology Department.

Statistical analysis: According to the data obtained during study period, descriptive statistics of somatic cell counts, milk yields, bacteriological isolations and identification were measured for the dairy farms on monthly, seasonal and annual basis. In order to test whether the seasonal differences between somatic cell counts of the farms and the average milk yield were statistically different, one-way ANOVA and Tukey's HSD tests from Post-Hoc tests were used. A Chi-Square Test was performed in order to do statistical comparisons in terms of seasonal bacteriological isolations. The results were tested at minimum 95% significance level. Statistical analyses were performed by using the SPSS® for Windows 14.01 (SPSS Inc., Chicago, Illinois, USA) (License No: 9869264) and STATISTICA®/7 package program.

Results

Average number of the cows for each farm was 175, and the total number of cattle in the farms was 24.150 during the period the study was performed. Some records were kept for the cows on all farms, but it was insufficient. All of the farms had free stalls and used natural bedding material (manure).


**Milk yield:** It was observed that the milk yield increased especially in spring term, and reached to its highest levels in May (Figure 1).

**Meteorological data:** All data (temperature, relative humidity, and rainfall data) were received from Northern Cyprus, Ministry of Public Works and Transportation, Meteorology Department are given in Table 1.

**Evaluation of the findings on somatic cell count:** BTMSCC median value was the highest during February (669,000 cells/ml), whilst it was at its lowest during November (474,000 cells/ml) with a monthly average of >400,000 cells/ml, annually (Figure 2).

The highest BTMSCC median value was during the winter months (596,166 cells/ml), and at its lowest level in autumn (479,333 cells/ml); and significant seasonal variations were seen in BTMSCCs ($P<0.05$). The average BTMSCC for all seasons was >400,000 cells/ml (Table 2).

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**Table 1. Monthly meteorological data received from Northern Cyprus, Ministry of Public Works and Transportation, Meteorology Department (2009-2010).**

| Average of the Lowest Air Temperature | Average Air Temperature | Average of the Highest Air Temperature | Average Relative Humidity (%) | Rainfall Averages (mm) |
|--------------------------------------|-------------------------|---------------------------------------|-----------------------------|-----------------------|
| October                              | 17.8                    | 22.8                                  | 28.4                        | 62.0                  | 35.5                   |
| November                             | 12.3                    | 16.7                                  | 21.5                        | 66.2                  | 41.9                   |
| December                             | 10.6                    | 14.3                                  | 18.5                        | 75.3                  | 154.5                  |
| January                              | 9.3                     | 13.0                                  | 16.8                        | 73.5                  | 112.0                  |
| February                             | 9.0                     | 12.8                                  | 16.7                        | 73.2                  | 158.0                  |
| March                                | 10.0                    | 14.8                                  | 19.6                        | 68.9                  | 7.6                    |
| April                                | 12.3                    | 17.7                                  | 23.0                        | 62.3                  | 12.7                   |
| May                                  | 16.1                    | 21.2                                  | 26.6                        | 63.3                  | 13.9                   |
| June                                 | 19.6                    | 24.7                                  | 29.8                        | 63.1                  | 7.6                    |
| July                                 | 22.2                    | 27.2                                  | 32.2                        | 66.2                  | 2.3                    |
| August                               | 24.6                    | 29.6                                  | 35.2                        | 62.5                  | 0.4                    |
| September                            | 21.5                    | 26.5                                  | 31.8                        | 61.1                  | 2.1                    |

Table 2. The seasonal median BTMSCC values (cells/ml).

| Season     | Median  | Max    | Min   | X (log) ± SEM | P   |
|------------|---------|--------|-------|---------------|-----|
| Autumn     | 479.333 | 1,524,000 | 158,000 | 5.69±0.02 |     |
| Winter     | 596.166 | 2,015,000 | 141,333 | 5.76±0.02 | <0.05 |
| Spring     | 575.666 | 2,143,000 | 127,666 | 5.74±0.02 |     |
| Summer     | 524.166 | 1,446,333 | 150,000 | 5.71±0.02 |     |

abc: The different letter in the same column symbolizes the difference.
abc: Ayni sütündaki farklı harf anlamılığı simgeler.
It was also determined that 74% of all farms had >400,000 cells/ml annually; and 67% of samples had >400,000 cells/ml. At the end of the study, annual mean BTMSCC was 521.583 cells/ml.

**Bacteriological isolation findings:** When bacteriological culture results were evaluated it was determined that the isolation was occurred in all samples (100%) in June. The lowest bacteriological isolation levels were seen in November (74.1%). Bacteriological isolation rates increased during periods of elevated temperatures, and decreased during times of lower temperatures (Figure 3).
Table 4. Monthly bacteriological isolation and identification findings.

| Bacteria                     | October | November | December | January | February | March | April | May   | June   | July   | August | September |
|------------------------------|---------|----------|----------|---------|----------|-------|-------|-------|--------|--------|--------|-----------|
| Coagulase Negative Staphylococci | 25.57   | 31.76    | 21.64    | 16      | 25.2     | 20.78 | 16.84 | 23.02 | 27.05  | 20.86  | 22.15  | 22.93     |
| *Bacillus* spp.              | 28.98   | 27.7     | 28.65    | 16.8    | 17.89    | 21.88 | 12.76 | 16.55 | 13.15  | 16.16  | 18.42  | 18.51     |
| *S. aureus*                  | 0       | 0        | 0        | 12.8    | 13.01    | 17.43 | 11.22 | 25.42 | 21.84  | 19.63  | 19.74  | 19.34     |
| *S. dysgalactiae*            | 0       | 0        | 0        | 0.8     | 0        | 2.49  | 30.1  | 14.87 | 20.84  | 15.75  | 13.6   | 11.33     |
| *S. uberis*                  | 1.14    | 14.86    | 12.28    | 8       | 4.07     | 24.93 | 6.12  | 5.52  | 4.22   | 5.32   | 4.61   | 8.29      |
| *S. agalactiae*              | 0       | 0        | 3.51     | 1.6     | 0        | 3.88  | 7.14  | 9.83  | 8.68   | 11.86  | 11.4   | 10.77     |
| *E. coli*                    | 16.48   | 8.78     | 9.36     | 0       | 8.13     | 8.59  | 13.27 | 4.8   | 1.24   | 8.38   | 7.02   | 8.84      |
| *Micrococcus* spp.           | 2.84    | 2.03     | 9.36     | 13.6    | 17.07    | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| *Pseudomonas* spp.           | 9.09    | 0        | 0        | 0.8     | 0        | 0     | 0     | 0     | 2.48   | 2.04   | 3.07   | 0         |
| *Enterobacteriaceae* spp.    | 9.09    | 0        | 0        | 12      | 0        | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| *Proteus* spp.               | 0       | 0        | 5.85     | 6.4     | 8.94     | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| *Aeromonas* spp.             | 0       | 0        | 7.6      | 0       | 5.69     | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| Yeast                        | 1.14    | 6.08     | 0        | 0       | 0        | 2.55  | 0     | 0.5   | 0      | 0      | 0      | 0         |
| *Pasteurella* spp.           | 0       | 8.78     | 1.75     | 0       | 0        | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| *Alcaligenes* spp.           | 0       | 0        | 11.2     | 0       | 0        | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
| *Corynebacterium* spp.       | 5.68    | 0        | 0        | 0       | 0        | 0     | 0     | 0     | 0      | 0      | 0      | 0         |
Seasonally, increasing bacteriological isolation rates in spring (88.8%) reached peak values in summer, and that the isolation rate was 98.3% in the samples. The isolation rate in summer was higher than that in winter and in spring \( (P<0.01) \). Similarly, isolation rates in spring were significantly higher than that in autumn or winter \( (P<0.05; \text{Table 3}) \). At the end of the study period it was determined that mean isolation rates were in 88.4% of the samples. Findings of bacteriological isolation and identification are shown in Table 4.

When bacteriological isolation and identification results are evaluated in terms of seasons CNS (25.51%), *Bacillus spp.* (23.18%) and *E. coli* (10.79%) were the microorganisms most abundant in autumn; *Bacillus spp.* (21.96%), CNS (21.00%) and *Micrococcus spp.* (12.89%) in winter; CNS (20.94%), *S. aureus* (19.61%) and *Bacillus spp.* (17.76%) in Spring; and CNS (23.15%), *S. aureus* (20.33%) and *S. dysgalactiae* (16.54%) in summer (Table 5).

After the cultures that were performed throughout the year it was determined that the isolation rates of CNS were highest with 22.73%; and *Bacillus spp.* (18.68%) and *S. aureus* (26.55%) followed CNS (Figure 4).

**Discussion and Conclusion**

Milk SCC in countries where animal breeding has developed is the most important criterion while evaluating milk quality (16, 31, 34, 42). The European Economic
Union Regulation 92/46 requires that the milk whose Somatic Cell Number is more than 400,000 cell/ml cannot be used as raw milk. The same regulation concluded that this type of milk was not suitable for human consumption as of 1998 (1, 8, 22, 33).

The results of various researches in different countries are shown in Table 6.

In this study, the average BTMSCC in Northern Cyprus throughout year was determined as 521.583 cells/ml (log 5.74 cells/ml). It was also found that 74% of the herds had BTMSCC levels>400.000 cells/ml. Results from this study are similar to other studies (2, 18, 37, 41). Therefore, BTMSCC levels being >400.000 cells/ml in our study show that there are important widespread udder health problems in many herds (BTMSCC>400.000 cells/ml in 74%) in Northern Cyprus, and that the mastitis control methods are of poor quality or are insufficient. These results led us to consider that subclinical mastitis levels are higher in dairy farms.

Our study revealed that BTMSCC levels in autumn, winter, spring and summer were 479.333; 596.166; 575.666 and 524.166 cells/ml in average, respectively; and that these levels showed significant variance according to the seasons (P<0.05). BTMSCC in autumn and summer were lower than that in winter and spring (P<0.05). Stulova et al. (36) reported that BTMSCC was the highest in June, and the lowest in November in 131 farms in Estonia. Similarly, in our study, it was determined that BTMSCC was the lowest in November, and the highest in winter months. The increase in BTMSCC during winter might be associated with the increase in clinical mastitis based on environmental factors and management malpractices. Norman et al. (19) reported that BTMSCC in the USA was lower from October to January (280,000-300,000 cells/ml), and higher in July and August (340,000 cells/ml). However, in study, whilst the BTMSCC was lower in autumn, it was at its highest levels during the winter and not in summer, which is different from the findings of the other studies.

Some authors (3, 7, 34) reported that BTMSCC has increased in summer. Ellis et al. (6) reported that the cleanliness scores of the cows were influenced negatively in seasons with increased levels of rainfall which resulted in subclinical mastitis and BTMSCC increased in herds. Reneau et al. (26) reported, when the cleanliness scores of udder and rear legs were lower SCCs also increased; and added that there was a strong correlation between the two situations. Valde et al. (40) conducted a study in Norway and reported, when the herd hygiene score was “perfect”, BTMSCC decreased by significant levels. This study has shown, while BTMSCC was >400.000 cells/ml in summer and winter, BTMSCC was significantly higher (P<0.05) in winter when compared to summer. The reason for this may be the fact that according to Northern Cyprus Meteorology

Table 6. Results of various researches in different countries.
Tablo 6. Farklı ülkelere yapılan çalışmaların sonuçları.

| Country                  | Number of herd | Average BTSCC of herds | Percentage of herds (BTSCC >400.000) |
|--------------------------|----------------|------------------------|---------------------------------------|
| Norman et al. (2000)     | USA            | 539.577                | 307.100 (in 1996) 313.500 (in 1997) 29 (1996-1997) |
| Rysanek and Babak (2005) | Czech Republic | 2769                   | 220.000 (in 2003) 12                         |
| Skrzypek et al. (2004)   | Poland         | 212                    | 296.000                                | 11.3                                    |
| Stulova et al. (2010)    | Estonia        | 131                    | 227.000 (2004-2007) 5                  |
| Elmoslemany et al. (2009)| Canada         | 235                    | 218.000 (2005-2007)                     |
| Holm et al. (2004)       | Denmark        | 5923                   | 270.000 (2000-2002)                     |
| Sampimon et al. (2008)   | Holland        | 200                    | 331.000 (in 1985) 219.000 (in 2007)       |
| Rysanek et al. (2009)    | Czech Republic | 268                    | 173.000                                |                                          |
| Kelly et al. (2008)      | Ireland        | 282.887                | 282.887                                |                                          |
| Wickström et al. (2009)  | Sweden         | 319                    | 392.220                                |                                          |
| Jayarao et al. (2004)    | USA (Pennsylvania) | 126                | 315.190                                | 41.2                                    |
| Van Schaik et al. (2005) | Chile          | 150                    | 408.000                                | 55                                      |
| Bouman et al. (2005)     | Uruguay        |                        | 546.100 (in 1996) 341.000 (in 2004)       |
| Miranda-Morales et al. (2008) | Mexico          | 112                    | 465.000                                |                                          |
| Suriyasathaporn et al. (2010) | Tailand      | 133                    | 412.000                                |                                          |
Department’s data there was a high rainfall in winter, especially in the year when this study was conducted, and this influences the hygiene scores of the cows in a negative way; and also it may be related to infection status in udders of cows.

Smith et al. (35) reported that seasons, lactation numbers and periods, and temperature stress increased SCC. By the results of bacteriological analysis in this study, it was determined that subclinical mastitis prevalence was higher in farms. High levels of BTMSCC during all seasons was thought to be associated with infection prevalence rather than a seasonal influence. Bulk tank milk cultures are among the most important methods that reveal milk quality and udder health (12, 14, 25, 27, 31). In this study, bacterial isolation was achieved in 88.4% of tank milk samples, while no isolation could be done in 11.6% of the samples. In tank milk microbiological cultures, bacterial isolation was done in autumn, winter, spring and summer with 83.2%; 83.4%; 88.8% and 98.3%, respectively.

The bacteria that causes mastitis are a potential source which can lead to the contamination of raw milk (21). It was determined in the samples that CNS was isolated at a rate of 22.73%, and Bacillus spp. 18.68%, S. aureus 16.55%, S. dysgalactiae 11.53%, S. uberis 8.14%, S. agalactiae 7.62%, E. coli 7.44%, Micrococcus spp. 1.81%, Pseudomonas spp. 1.49%, Enterobacteriaceae spp. 0.90%, Proteus spp. 0.85%, Aeromonas spp. 0.58%, Yeast 0.53%, Pasteurella spp. 0.47%, Alcaligenes spp. 0.41%, Corynebacterium spp. 0.29%. CNS, which were isolated at the highest rates in this study, are the microorganisms that are dominant in many countries as Taponen and Pyörälä (38) stated.

Recently, CNS has been the most common pathogen isolated in subclinical mastitis in many countries (38). It has been reported recently that there is a clear increase in isolation rates of CNS species from mastitic milk, and that the prevalence varies between 10% and 50% (23, 28, 39). Coagulase Negative Staphylococci are opportunistic pathogens and have the opportunity to grow in the bacterial flora of teat skin. Given the opportunity for growth, they can reproduce rapidly and lead to mastitis (13).

Tenhagen et al. (39) conducted a study in Germany and reported that 35% of udders with subclinical mastitis were infected with CNS. Roberson et al. (28), conducted a study in Tennessee, the USA, and reported that in herds with high SCC, CNS prevalence was 12-41% and average CNS infection rate was 28%. Makovec and Ruegg (17) conducted a study in Wisconsin in the USA and reported that CNS rates in milk with subclinical mastitis increased to 17.5% from 12.7% between years 1994 and 2001. Poelarends et al. (24) reported that they isolated CNS in 6% of udders from herds with high SCC levels in Netherlands. Dingwell et al. (5) reported that 15% of new intra mammary infections after parturition occurred due to CNS in both the USA and Canada. Davidson et al. (4) reported that CNS infection prevalence in early lactation periods in Canada was 5-6%, and in proceeding lactation periods, this rate varied between 14-17%. Haltia et al. (9) conducted a study in Estonia and reported that CNS rate was 16%. Pitkälä et al. (23) conducted a study in Finland and reported that prevalence of CNS infection was high and that they isolated CNS from 50% of the samples with bacterial growth. Østerås and Solverod (20) conducted a similar study in Norway and reported that CNS prevalence was 16%. In this study, 22.7% CNS was isolated from bulk tank milk samples. This result is lower than those of Pitkälä et al. (23) and Roberson et al. (28), and higher than those of other authors. The fact that the most frequently-isolated bacteria in Northern Cyprus is CNS may stem from the mastitis control methods being applied by some farms and therefore contagious pathogens being under control.

The study is the first national report that investigated prevalence of mastitis pathogens, mastitis incidence and herd-level SCC within a year in Northern Cyprus. BTMSSCs were high throughout the year in dairy farms in Northern Cyprus and this varied according to the season. The most frequently isolated pathogens were CNS, Bacillus spp. and S. aureus. In conclusion, the results of our study clearly shows that there are important problems in terms of udder health on dairy farms in the Northern Cyprus. Our study gives important information about herd-level of mastitis, SCC in bulk tank milk and prevalence of pathogens causing mastitis that can be useful for veterinarians, advisors and farmers. However, further nationwide studies are needed to determine risk factors for mastitis in Northern Cyprus.

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